# Split Sampling Study for the Maryland and Virginia Mesozooplankton Monitoring Programs

Final Report, June 2000

ICPRB Report 00-3

Prepared by

Interstate Commission on the Potomac River Basin Suite 300, 6110 Executive Blvd. Rockville, Maryland 20852

for

United States Environmental Protection Agency Chesapeake Bay Program 410 Severn Avenue Annapolis, Maryland 21403

#### **Forward**

A draft report of the 1998-1999 Mesozooplankton Split Sample Study was compiled by the Interstate Commission on the Potomac River Basin in April, 2000. The draft report was reviewed by the state monitoring program managers, principal investigators and staff of the zooplankton monitoring programs, and representatives from the Chesapeake Bay Program Monitoring and Living Resources subcommittees. Comments and recommended changes from reviewers were documented in a tracking sheet and specific changes to the draft report ("actions") were proposed. The tracking sheet and proposed changes were submitted for review and approval to the Monitoring Subcommittee Coordinator and the program managers in the Virginia Department of Environmental Quality and Maryland Department of Natural Resources. Approved changes were then implemented in this final report. Uncontested sections were also edited to condense or clarify text.

#### ICPRB Report 00-3

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#### **Executive Summary**

Laboratory methods of the Maryland and Virginia mesozooplankton monitoring programs had not been compared before this 1998-1999 Split Sampling Study, however state managers and laboratory staff were aware that method differences were affecting the monitoring results. The programs implemented modifications to their laboratory counting protocols in 1998 in order to better estimate species richness in Maryland and eliminate laboratory sieving losses of smaller mesozooplankton taxa and life stages in Virginia. The goal was to make Chesapeake Bay mesozooplankton counts in the two states directly comparable. The 1998 - 1999 Split Sample Study indicates the desired outcomes of the modifications were only partially accomplished. The "new" Versar counting method (Maryland program) has improved Versar's ability to measure species richness, an important Bay-wide indicator, and the "new" ODU counting method (Virginia program) has increased ODU's taxa counts per sample. However, the "new" ODU method still produces significantly lower total counts than the Versar method. The method consistently counts less of certain taxa, particularly the immature (copepodite) life stage of calanoid copepeds which are a common and frequently dominant taxonomic group. Sample variances in counts produced with the "new" ODU method are higher than sample variances in counts produced with the Versar method, hence the ODU estimates of precision are lower. Finally, the number of taxa identified per sample was on average lower in the "new" ODU counts.

A single method needs to be selected and implemented because the modified laboratory methods of the two programs do not produce comparable results. While program principal investigators feel the existing monitoring data provide meaningful status and trend assessments within each state, a single method will ensure that Maryland and Virginia results are comparable bay-wide. It will allow the CBP monitoring programs to calculate and use a diverse suite of bay-wide mesozooplankton indicators and more effectively address the information needs of the Program. Bay-wide zooplankton community indicators are needed because they are useful tools in measuring overall ecosystem health, targeting restoration efforts in open water habitats, and tracking food web responses to management actions such as nutrient and sediment reductions.

The Split Sample Study identified other procedural problems that need to be resolved. There appears to be within laboratory and between laboratory differences in taxonomic identifications. These differences could be reconciled with side-by-side comparisons and the assembly of a photographic or archival specimen collection for Chesapeake Bay mesozooplankton. Quality assurance procedures should be maintained in each laboratory to ensure adequate taxonomic training of new technical staff. Quality assurance (repeated) counts for each laboratory should be regularly submitted to the states, the Chesapeake Bay Program or their designees for independent analysis. Regular site visits between the two states' technical staffs should be carried out to ensure comparable interstate taxonomy. A split sample study should be done annually for at least the next few years to ensure interstate count comparability.

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# Split Sampling Study for the Maryland and Virginia Mesozooplankton Monitoring Programs

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#### Introduction

The Chesapeake Bay Monitoring Program has included a plankton component since it began in 1984. The current Maryland and Virginia zooplankton programs are partially or mostly funded through the CBP Living Resources Subcommittee. Old Dominion University (ODU) collects and counts mesozooplankton for Virginia Department of Environmental Quality (VADEQ); Versar, Inc. collects and counts mesozooplankton for Maryland Department of Natural Resources (MDDNR). While sample collection methods in the field are reasonably comparable, discrepancies were suspected in the mesozooplankton data from the start because the laboratories began their monitoring programs using different laboratory analysis methods (see Appendix A and documentation on-line at <a href="http://www.chesapeakebay.net/">http://www.chesapeakebay.net/</a>). The ODU and Versar laboratory methods had not been directly compared before the 1998-1999 Split Sampling Study.

Versar employs a variation of a commonly used counting technique of subsampling using the Stempel pipette method. The Versar method dilutes samples to a standard volume (e.g. 800 mls) and counts subsamples until the requisite number of organisms has been counted to attain  $\pm 20\%$  precision for the total count. This method is known to less accurately count the rarer species of zooplankton. Early in the program, Versar also scanned the entire sample at low magnification and counted all larger, rarer forms. The laboratory dropped this effort in 1989 due to budget constraints, but instituted a hierarchical counting modification which produces better counts of subdominant species. An error level of at least 25% is presently obtained for the dominant and subdominant taxa while a level of  $\leq 20\%$  is maintained for the total count.

ODU employs a modification of the innovative Controlled Variability Sampling (CVS) method which is intended to reduce the variance in counts of the larger, rarer forms (Alden et al. 1982). Samples are filtered through the CVS apparatus which consists of a stack of differently sized sieves that sort zooplankton individuals by size ranges. Organisms on each sieve are washed off the sieve and repeatedly split with a Folsom Plankton Splitter until the number of organisms has been reduced to a level where an entire split can be counted. This method is designed to more accurately count the larger, rarer forms. An error level of 35% was chosen for both common and rare species of interest. The CVS method used in the ODU monitoring program was different from the method originally described in Alden et al. (1982) in an important way: the monitoring program used a series of 2000, 850, 600, 300, and 200 micron sieves while the original method used a series of 2000, 850, 600, 300, 150, and 75 micron sieves. A percentage of mesozooplankton taxa was suspected of being lost by the monitoring program CVS apparatus because its bottom sieve (200 microns) was the same mesh size as that of the ODU plankton nets used to collect mesozooplankton samples in the field. Long, narrow mesozooplankton such as copepods are know to pass through 200 micron mesh plankton nets and sieves (e.g. Edmondson and Winberg 1981, Harris et al. 2000).

Recent efforts to develop and apply bay-wide zooplankton indicators of ecosystem health highlighted the data discrepancies. Program principal investigators felt that status and trend analyses of the monitoring data *within* each state were valid and provided good information in spite of the methodology biases. However, the application and use of many potential bay-wide indicators were suspect because Maryland and Virginia data sets did not appear to be comparable. The Chesapeake Bay Program needs bay-wide zooplankton community indicators because they are useful tools in measuring overall ecosystem health and targeting restoration efforts in open water habitats (status), and tracking food web responses to management actions such as nutrient and sediment reductions (trends and linkages). Mesozooplankton indicators will soon be used to measure CBP progress in attaining plankton restoration goals. Before bay-wide indicators can calculated and used with any confidence, the Maryland and Virginia mesozooplankton monitoring data must be made comparable.

After long-running discussions and several meetings, the ODU and Versar mesozooplankton monitoring program staffs met in January 1998 at ODU for a side-by-side comparison of counting techniques. The comparisons showed that Maryland protocols insufficiently measured mesozooplankton species richness because they were not counting large, rare taxa (e.g. *Neomysis americana*, *Rithropanopeus harrissii*). Virginia protocols counted significantly lower abundances of major mesozooplankton species (Table 1), especially for the sole tidal freshwater sample where the ODU total count was less than 1% of the Versar total count.

The Maryland and Virginia programs agreed that modifications to their current laboratory methods might resolve the discrepancies. The laboratories recommended specific changes to improve comparability. The "new" method modifications would give the programs both backwards <u>and</u> forwards compatibility in both states. This was the desired outcome from the management and data analysis perspectives. The states would not lose data for long-term trend analyses (backward comparability), and they would have direct comparability in the future (forward comparability). Regular split sampling would be used to document that this "performance-based approach" was successful, i.e. different methods were producing the same results.

The proposed modification were as follows:

ODU staff would continue to use the customary Controlled Variability Sampling (CVS) apparatus. They would attach a 72 micron sieve to the bottom of the CVS apparatus in order to capture smaller-sized individuals which had previously been washed through the CVS system into the sink. ODU would obtain an "old method count" using data collected from the CVS original sieves and a "new method count" by combining enumerations from the old method and the 72 micron sieve.

Versar would add a step to its usual subsample counting method. After completing its standard protocol and obtaining an "old method count," Versar would filter the whole sample through a large-size (850 micron) screen to concentrate and enumerate the rarer, large-sized individuals. Versar would obtained a "new method count" by combining enumerations from the old method and the large-size sieve.

Calculations of species densities that *include* the additional "patch" counts are intended to make the mesozooplankton results from the two laboratories directly comparable. If split sampling shows that they were, the "new method counts" would be used in the future to calculate bay-wide

indicators. Calculations of species densities that do not include the additional "patch" counts would allow both laboratories to maintain backward compatibility with the historical data in each state and continue to determine long-term trends.

In July 1998, ODU ended efforts to measure mesozooplankton biomass (dry weights and ash-free dry weights) and began counting the additional, 72 micron sample fraction. Versar had already dropped its laboratory measurements of biomass and had begun counts of the sample fraction caught in the large-sized sieve. A split sample study was needed to confirm that the new methods were working as intended.

#### Split Sample Project - Round 1

A split sample project was proposed to the Monitoring Subcommittee in the Spring of 1998, and funds were made available to the contractors to enumerate split samples. The "new method counts" for mesozooplankton were intended to demonstrate the new methods' comparability. Split samples were collected in April, May and June of 1998. The Virginia and Maryland laboratories each collected 12 mesozooplankton samples during their regular monitoring cruises. The preserved samples were split in half. One split was enumerated by the originating laboratory as part of its monitoring program, and the other was enumerated by the corresponding lab in the other state. Unless otherwise noted, the counts produced for each split sample were enumerations of all taxa in the sample, identified to the usual taxonomic level. The sites investigated included locations in a range of salinities, with exposure to different river basins and environmental conditions. Two sets of counts were produced by Versar and ODU for each split sample: one count generated with the laboratory's old method and one generated with their modified method. Specifically, Versar produced a count with its original method and a count which included enumerations of mesozooplankton caught on the added 850 micron sieve. ODU produced a count with its original CVS method and a count which included enumerations of mesozooplankton caught on the added 72 micron sieve. Mr. Mateja, Mr. Crock, and Mr. Miebert, the three ODU mesozooplankton laboratory staff, all participated in counting the 24 Virginia split samples. Mr. Craig Bruce of the Versar staff counted the 24 Maryland split samples. All split sample enumerations were completed in the late summer of 1998, and the results were forwarded to the CBP Quality Assurance Officer and to the Interstate Commission on the Potomac River Basin for analysis. Commission staff sent the raw data to Elgin Perry (statistician) for analysis and also calculated a suite of mesozooplankton indicators (Table 2, 3).

The results of this first set of mesozooplankton split sample counts ("Round 1") were discussed at the "Plankton Summit" meeting<sup>2</sup> and in a subsequent conference call. It was concluded that the Round 1 mesozooplankton results were mostly invalid due to a malfunction of the modified

<sup>&</sup>lt;sup>1</sup> The author of this report was under the impression that the ODU laboratory supervisor, George Mateja, was the sole counter of the Virginia split samples in Round 1 and listed him as such in the minutes of various conference calls and in the report's draft version. The ODU Principal Investigator indicated in his review that all three of the ODU mesozoop lankton laboratory staff participated in counting the Round 1 split samples.

<sup>&</sup>lt;sup>2</sup> A meeting of the plankton monitoring program principal investigators, staff, managers and data analysts was held at Old Dominion University on September 11-12, 1998. Dubbed the "Plankton Summit" by participants, the purpose of the two-day meeting was to review the initial results of the phytoplankton, microzooplankton and mesozo oplankton split sample studies. Participants were also given a tour of the ODU plankton lab oratories.

CVS method at ODU and other problems. The motorized siever of the CVS method originally had a stack of 2000, 850, 600, 300, and 200 micron sieves, and an additional sieve chamber with a size of around 75 microns was added to the bottom of the sieve array in order to capture and count the smaller mesozooplankton taxa. The modified apparatus appeared to function normally while the first round of splits was carried out. However, after the Plankton Summit, ODU staff realized that zooplankton were being forced out of the sides of the smallest, added sieve chamber. The normal tolerances that worked for the other sieve chambers were not working between the 75 micron and 200 micron chamber because of increased water pressure in the 75 micron sieve chamber. The problem could be fixed by removing the 75 micron chamber and adding a 63 micron passive sieve placed underneath as a catch basin for discharge water. There were additional problems with Round 1 of the split sample study that cast doubt on the validity of the results. Six splits counted by ODU (five collected by Versar, one by ODU) were in a state of decomposition when they were processed for sampling by ODU. The laboratory sheets note "poorly preserved" on the samples. Also, ODU's original electronic data submittal contained many data entry problems, so a number of iterations of the data developed as these errors were caught and corrected. The processed data (e.g. indicators) produced by ICPRB are probably accurate for the most part however they were never closely checked against the raw data sheets to confirm that all the errors were caught.

Despite the CVS method malfunction, the poorly preserved samples, and the data entry errors, there are a few general conclusions that can be drawn from some of the data:

- Species richness was higher in the modified Versar method, indicating the Versar modified method, or "patch," was working.
- Many of the split counts had percent differences greater than ± 20%, suggesting a high degree of variability in one or both laboratories.
- The QA/QC approaches in the two laboratories did not produce comparable measures of the variance in replicate counts and hence could not be used to compare the two laboratories.
- There appear to be taxonomic differences in the counts produced by the two laboratories. Specifically:

Temora longicornis vs. T. turbinata

Cyclops vernalis vs. Anthocyclops vernalis

Eurytemora affinis vs. Eurytemora hurinoides

Polyhaline species *Temora longicornis* identified at tidal fresh and oligohaline stations by ODU

RET3.1 differences in Cladocera

The differences in the ODU and Versar "new" method counts continue to prevent bay-wide application of most of the indicators developed for mesozooplankton to characterize health of the zooplankton community in Chesapeake Bay.

The following recommendations were made during the "Plankton Summit:"

- <u>Recommendation</u>: A more thorough statistical analysis of the split sample data should be performed.
- Recommendation: The laboratories should institute a regular split sample program.
- Recommendation: If future split sample counts aren't comparable, a microspheres (beads) experiment could be done to compare the lab methods using known quantities of

- different sized beads. Alternatively, the method could be tested on a prepared sample of known quantity and species composition.
- <u>Recommendation</u>: The laboratories should resolve taxonomic issues that seem to be occurring between laboratories.
- Recommendation: The historical data should be corrected to reflect the taxonomic regrouping, changes, etc. made in resolving the above taxonomic issues. This will involve resubmittal of the data.
- <u>Recommendation</u>: Several taxa are counted by both the micro- and mesozooplankton programs. The group made the following decision:

Bosmina, barnacle nauplii and polychaete larvae counts from the mesozooplankton program data should be used for purposes of calculating bay-wide indicators.

Pelecypod larvae, rotifer, and copepod nauplii counts from the microzooplankton program data should be used for the bay-wide indicators.

Individual programs should count whatever is in their samples if they want to and put those numbers in their own databases, but should include only the appropriate taxa in the databases they submit to the CBP Data Center.

<u>Recommendation</u>: Provided there are funds, ODU should start to do complete replicate sample counts as part of an additional QA/QC procedure to check precision. The method will be similar to the procedure currently used by Versar. This would involve an additional sample count per month. ODU's old QA/QC method of counting both pairs of a split for one of the seives may or may not be continued.

# Split Sample Project - Round 2

The ODU mesozooplankton monitoring program proposed redoing the split sample counts after further modifying the CVS method to overcome the leakage problem caused by the addition of the 75 micron sieve. They would: a) return to using the originally sieves (i.e. 2000, 850, 600, 300, and 200 u mesh) and approach, b) funnel the water washing through the CVS apparatus into a large diameter, 64 u mesh sieve as it drains into the sink, and c) count all of the original sieve fractions and the additional 64 u sieve fraction. The ODU laboratory could perform recounts on some or all of their original split samples (12 from Versar, 12 from ODU) because a) Versar archived its 12 split samples after counting them and could provide them to ODU, and b) ODU archived splits of its original split samples and could recount them. Measures could be taken to ensure the counts are done "blind."

Funds within an existing grant to the Interstate Commission on the Potomac River Basin (ICPRB) were reallocated to provide funds for ODU to recount splits. The planned statistical analysis of the questionable Round 1 split samples was discarded, and Dr. Elgin Perry (statistician) agreed to analyze the new split sample results ("Round 2") as they were produced. A contract was set up between ICPRB and Mr. Forrest Crock, the ODU technical staff member designated by ODU Principal Investigator Kent Carpenter to do the recounts. Assurances were made that Mr. Crock had been trained by Mr. Mateja, the ODU laboratory supervisor, and produced comparable counts.

In Round 2, ten archived split samples from Round 1 were recounted by ODU, then a side-by-side taxonomic comparison was performed by the staff of the two programs, and finally ten new split samples were counted by both Versar and ODU. Round 2 results were reviewed after the

first five splits, the second five splits, and the final ten splits. Adjustments were made to the original Round 2 scope of work at these times as issues developed or were resolved. The results of the first ten (archived) split samples are not directly comparable to the second ten (new) split samples because the laboratories made corrections in taxonomic identifications midway. Furthermore, ODU made changes in life stage identification procedures between the first five and second five splits which affected comparisons of adult and copepodite life stage abundances of certain copepods. Attention therefore is focused on the last ten split sample results in this report section, although major points from the first ten split samples are presented.

### Round 2 - First Ten

Results of the ODU recounts of ten archived split samples ("first ten") were matched by ICPRB with their Versar counterparts (enumerated earlier as part of Round 1). In order to remove analysis differences caused by laboratory differences in level of taxonomic identification, ICPRB staff reviewed the species list and inserted an additional, adjusted NODC code for each count ("newNODC"). Species names or NODC codes that were closely related but not identical could then be matched on this new field. In many cases this involved backing species identifications to a higher taxonomic level (e.g. making "Acartia sp." and "Acartia tonsa" equivalent to "Acartia"). The data and data documentation were forwarded to Elgin Perry for statistical analysis. The following steps were taken by Dr. Perry in analyzing the split sample results. The procedures are described in more detail in Appendix B.

- Process the data: All raw counts within sample that have the same NEWNODC code were summed. The estimated taxon totals were recomputed from the raw data (this confirmed that Perry and ICPRB handling of the taxonomic data produced identical results). The estimated total count and sampling variance for each sample-taxa-lifestage (and sieve in the case of ODU) was computed. (For ODU, estimated counts and their variances were summed across sieves.)
- Coefficient of Variance: Univariate analysis was done on the difference between the Versar coefficient of variation and the ODU coefficient of variation for taxa-lifestages identified in both split samples. Variable = CVDIFF = Versar Coefficient of Variation minus ODU Coefficient of Variation. (Table 4).
- Z-Score: A z-score was computed using the variances in order to compare the labs on a sample by sample/taxa by taxa basis.
- Wilcoxon Signed Rank: A Wilcoxon signed rank analysis was done on all samples for each taxa. (Table 5).

The results were discussed by the zooplankton monitoring program staff in two conference calls. A summary of the observations and decisions is presented below:

• Copepod life stage enumerations Differences in Versar and ODU life stage counting procedures in laboratory were apparent in the original Round 1 counts and the first five recounts. They prevented direct comparisons of copepod results. Specifically, ODU enumerated copepodite life stages of just of Acartia, Eurytemora, and Mesocyclops while Versar enumerated copepodites of all copepods. Copepodites that are not identified as such in the ODU samples are automatically grouped with the "adult" category when the ICPRB indicator calculations are run. Therefore, the "adult copepod" category inadvertently contained copepodite numbers in the ODU results. ODU enumerated copepodites of all copepod species in counts after the first five split samples recounts.

- *Taxonomic identifications* There appear to be recurring differences between Versar and ODU identifications of some species.
- Individual taxa abundances The results of the Wilcoxon Sign Rank statistical test (Table 5) seem consistent with the sample by sample comparisons in that when the p-value for the signed rank analysis is small, the individual sample analyses show a preponderance of differences in one direction with at least some of them significant. As Dr. Perry pointed out at the Plankton Summit, small raw counts can have a big and sometimes arbitrary impact on split sample outcomes, and often do not accurately represent sample contents. Taxa comparisons that include small raw counts should probably be ignored in these ten split samples.
- Total copepod and total mesozooplankton count comparisons Counts for total mesozooplankton and total copepods, two general groupings of the data, are not affected by the small raw count issues above. Versar counts for these taxonomic groupings are usually higher than the equivalent ODU counts (Figure 1).
- Taxa richness and diversity On average, Versar identified more unique taxa per sample than ODU (Table 6). Versar may be finding more small-sized mesozooplankton taxa such as ostracods, Alona, chydorids, Saphirella, and Cyclops vernalis but raw counts of these taxa are often low and are therefore not as reliable.
- Coefficients of variation The coefficients of variation for the ODU counts are noticeably higher than the Versar coefficients of variation (Figure 2, Table 4).

The results suggest the ODU "patch" (i.e. addition of small mesh screen positioned below the CVS stacked sieves) is partially correcting the original loss problem but the Versar-ODU counts are not directly comparable and other issues—primarily taxonomic—need to be addressed.

### Side-by-side review of Chesapeake Bay mesozooplankton taxonomy

The monitoring programs agreed that before more split samples were counted, the technical staff of the monitoring programs should meet and resolve the taxonomic differences apparent in the ODU/Versar split samples. Versar staff Craig Bruce traveled to ODU on March 10, 1999 and met with ODU staff Forrest Crock for two days. On March 12, 1999, they were joined by the George Mateja and Conrad Miebert, ODU, and Claire Buchanan, ICPRB, to review their findings. These findings were also summarized by the Versar staff after the meeting (Appendix C). Briefly,

- The laboratories agreed that annual meetings of the technical staff to discuss taxonomy and laboratory counting techniques should be continued to ensure comparability and allow the continued development of bay-wide zooplankton indicators.
- The laboratories agreed to stop including counts of rotifers since the microzooplankton programs generate more accurate numbers for this microzooplankton group.
- The laboratories agreed to identify to the lowest taxonomic level (e.g. *Gammarus* instead of unidentified amphipod) when possible in order to avoid inter-laboratory differences related to level of taxonomic identification.
- Versar technical staff previously misidentified barnacle cypris as ostracods at high salinity stations.
- One ODU staff previously misidentifying *Eurytemora* as *Temora* at some freshwater stations.
- ODU previously misidentifying *Eurytemora affinis* as *E. americana*.
- It appears that *E. affinis* and *E. hirundoides* are now considered to be synonymous.

- Nomenclature changes such as *Cyclops vernalis* to *Acanthocyclops vernalis* and *Cyclops bicuspidatus* to *Diacyclops thomasi* were discussed.
- To maintain consistency between the laboratories, it was agreed that:

The most common species of Daphnia will be identified to species level.

The most common species of Harpacticoid will be identified to genus and/or species level.

The most common Diptera will be identified to family or genus.

The most common Amphipod will be identified to family or genus.

Crab zoea and megalops will be identified to species level.

Specific larval stages (e.g. trochophore and spionidae) will not be differentiated. Instead they will be reported as polychaete larvae.

• The absence of *Bosmina longirostris* in the ODU and Versar Round 1 WE4.2 counts and its strong presence in the ODU Round 2 WE4.2 count was discussed. *Bosmina*, a freshwater species, is not found at mesohaline stations such as WE4.2. ODU felt the *Bosmina* count may have come from sample contamination during ODU sample sieving/splitting procedures.

Concerns about possible contamination of the split samples remaining to be recounted by ODU lead the group to agree to finish the split sample study with ten new samples. These same concerns also raised the issue of whether or not the Round 2 First Ten results and conclusions were tainted by contaminated split samples.

#### Round 2 Last Ten

Ten samples from the regular Maryland monitoring program (five from March, five from April) were used to avoid additional costs to the study. The samples were sent to ODU for counting after they had been counted and reconstituted by Versar staff. The split sample results were received by ICPRB staff in June, 1999, merged and sent to Elgin Perry for statistical analysis. The results (Table 7, Appendix D) and additional analyses provided by ICPRB (Table 8) were discussed in an October 19, 1999, conference call and in subsequent phone calls and emails. Briefly,

- Total mesozooplankton count comparison Differences between the Versar and ODU total mesozooplankton counts for individual split samples were greater than +20% in 9 out of 10 (90%) split samples, indicating Versar usually counted larger numbers of organisms in the split samples (Figure 3).
- Individual taxa abundances Sample-by-sample comparisons of taxa identified by both laboratories show that nearly a quarter of the z-scores (23%) are greater than 2.0 (i.e. Versar counts are significantly larger than ODU counts) while 11% of the z-scores are less than 2.0 (i.e. ODU counts significantly larger than Versar counts) (Appendix D). When the p-value for the Wilcoxon Signed Rank analysis is small (Table 7), the individual sample analysis show a preponderance of differences in one direction with at least some of them significant (Appendix D). In some cases, large sign rank differences are the result of the laboratories still identifying taxa to different taxonomic levels (lumping-versus-splitting). For example:

"Balanidae" vs "Balanus"

"trochophore" (ODU) and "polychaete" (Versar)

harpacticoida (Versar) vs Canuella elongata, Euterpina acutifrons (ODU)

• *Pooled data* In order to circumvent the high variance and/or low counts for some taxa, count data from the ten split samples were pooled to obtain "number per 10 samples."

The pooled data show that several species and taxonomic groups have similar counts (i.e. # per 10 samples) and the % difference for these pooled counts are less than  $\pm 20\%$  (Table 8). These species include Eurytemora adults, Acartia adults, Podonidae, and Bosmina. The Wilcoxon Rank Sign test supports these results (Table 7) although it suggests that differences in the *Eurytemora* adults counts are borderline significant (p<0.0840). Versar has higher pooled counts for each of the three general taxonomic groupings (Table 8): total Cladocera +39.9%, total Copepods +69.5%, miscellaneous +38.6%. Examination of the pooled data (Table 8) suggest the smallest body sizes and the narrowest body shapes may be the most affected, i.e. they have the largest percent differences. These include a) all small, round-bodied mesozooplankton without large spines (i.e. chydorids, barnacle cypris, ostracods) +61.2%, b) barnacle nauplii (these are tri-cornered and spiny but can be very small) +77.8%, and Acartia copepodites (minus their antennae, these are small- to medium-sized, narrow taxa) +76.8% Counts of Eurytemora affinis copepodite, a medium-sized life stage of a common and important copepod, were significantly different in the split sample results, with Versar counting approximately 3.8 times more individuals that ODU, for a percent difference of 116% (Table 8).

The possibility of both the ODU and Versar methods biasing counts of this species life stage was discussed and tentatively discounted (why would *Eurytemora affinis* copepodites be affected by a particular method but not the copepodites of other copepod species?).

- Coefficient of variation The coefficients of variation in the ODU taxa counts were again larger than those for the Versar counts, indicating ODU estimates of precision are lower than those of Versar (Figure 4).
- Taxonomic identifications Some differences that may be the result of conflicting taxa identifications. These possible identification differences are evident when ODU counts of taxa within a larger taxonomic group are higher than Versar counts while Versar counts for the whole group are higher than ODU counts (Table 8). For example,

ODU counts more "other Calanoid copepods" than Versar while Versar counts more total Calanoid copepods than ODU

ODU counts more "Cyclopoid" copepods than Versar while Versar counts more "total Copepods" than ODU

ODU counts more "other Cladocera" than Versar while Versar counts more "total Cladocera" than ODU

Potential identification differences are also seen when species by species comparisons are made and non-rare species that are found by one laboratory are never found by the other. For example,

Taxon	ODU total/10 splits	Versar total/10 splits
Alona (cladocera)	17,408	0
Ilyocrptus spinifer (cladocera)	0	75,200
Pseudocalanus copepodites (copepod	306,048	0

Although different in some ways from the Round 2 First Ten results, the Round 2 Second Ten results generally confirmed the earlier conclusions.

#### Round 2 Last Ten Followup

Several action items intended to complete the analyses or follow-up on the findings were recommended during the conference call and afterward:

- Check calculations. George Mateja, Forrest Crock, Craig Bruce and Claire Buchanan checked the various spreadsheets to determine if any correction factors, and especially those for *Eurytemora affinis* copepodites, were incorrect in the originally submitted results or the analyzed results. ODU found no errors in their split sample database while Versar staff found one error. The *Eurytemora affinis* copepodite count in one Versar split sample (Station TF1.5, 3-22-99) had a subsample volume of 2 where it should have been 1. Therefore, the Versar count for this split sample underestimated *Eurytemora affinis* copepodites, as well as total copepods, total mesozooplankton. This correction further accentuated the differences between ODU and Versar counts.
- Taxonomic groupings. Elgin Perry made comparisons of specific taxonomic groupings. These analyses were intended to circumvent taxonomic identification issues (i.e. level of taxonomy, different identifications, different life stage) and demonstrate whether or not the two methods are capturing and counting the same numbers of similar shaped/sized critters. Results are shown in Table 9.

Selected Copepods (all adults and copepodites minus Eurytemora copepodites). Nine out of the ten samples show significant  $(-2.0 \le z \ge +2.0)$  differences in counts. While the large z-scores do *not* show a preponderance in the positive or negative direction, they indicate that variance is unusually high in this grouping. Note: z-scores are similarly high for the common Eurytemora copepodites (calanoid), the one taxa excluded from the "selected copepod" grouping (Appendix D). However, for Eurytemora copepodites there is a preponderance of positive signs meaning Versar consistently had higher counts. Acartia copepodites (calanoid), a common taxon that was included in the selected copepods comparison, also shows a preponderance of positive signs when analyzed separately (Appendix D).

Polychaetes AND Trochophores. Most z-scores were non-significant indicating that the Versar and ODU counts are similar. Differences observed earlier are apparently due to the use of different life stage codes/names.

Round organisms (all cladocerans plus some of the miscellaneous group, including ostracods and barnacle cypris but excluding barnacle nauplii). Most z-scores were non-significant indicating that the Versar and ODU counts are similar. This result juxtaposed on the sharp differences observed for individual taxa within this grouping such as chydorids, Daphnia, "other Cladocera," and ostracods (see Table 8) suggests that there are still taxonomic identification differences between some of the categories.

Barnacle nauplii. Half of the samples show count significant differences (z > +2.0) with a preponderance of positive signs meaning Versar consistently had higher counts.

• Taxonomic identifications.

Specimen Archive. Each laboratory is beginning to assemble a reference collection of all the species encountered during regular sample analyses. Versar, for example, is "picking" 2 or more individuals of each species (and sex if possible) and preserving them in sample vials. This could eventually become a long-term reference collection to be compared and shared by both laboratories.

Meeting. Representatives of both laboratories should at some point meet and do a side by side comparison of their reference collections. Species identifications that cannot be resolved or that are in question will be submitted to outside experts for analysis.

List of experts. Laboratories will send to Claire Buchanan a list of experts in taxonomic identifications.

- Correction factors. Claire Buchanan reviewed a selection of the split sample results to determine if conversion factors could be used on the older, "pre-patch" ODU and Versar data for the purpose of calculating Bay-wide indicators (Table 8). The usefulness of the conversion factors appears doubtful given a) the taxonomic discrepancies between the states, and b) analysis results of the actual monitoring data (see discussion).
- Implement regular split sample comparisons as approved CBP funds become available. Joe Macknis (EPA) has indicated that the Chesapeake Bay Program would like to see plankton split sample counts done as soon as possible and has orchestrated the monitoring funds to allow this to happen. A critical issue evident in the previous split sample results is the apparent differences in taxonomic identifications. A possible use of the split sample allotment this year would be for ODU and Versar monitoring staffs to focus solely on resolving taxonomic issues rather than performing standard split sample counts.

#### Discussion

Analysis of the Round 2 mesozooplankton split sample results indicated that the desired outcomes of the laboratory method modifications were only partially accomplished. The ODU total mesozooplankton counts are, on average, still lower than Versar's and the ODU method appears to selectively undercount key taxa, particularly the immature (copepodite) life stage of calanoid copepods and small-sized taxa. The study also raised several unexpected issues: taxa richness is lower in the ODU samples, and the species lists and level-of-taxonomy are not identical between the two laboratories.

#### Selecting a Method

A fundamental requirement of the mesozooplankton monitoring data is that the data be directly comparable in order to meet present and future management needs. Representatives of the CBP mesozooplankton monitoring programs all acknowledged that a "performance based" approach was not possible with the modified Versar Stempel pipette method and the ODU "new" CVS method. In other words, the two laboratories could not use their different methods to produce directly comparable results. A single enumeration method needs to be selected and implemented. A single method will ensure that Maryland and Virginia results are comparable bay-wide. It will allow the CBP monitoring programs to calculate and use a diverse suite of bay-wide mesozooplankton indicators. Bay-wide zooplankton community indicators are needed because they are useful tools in tracking food web responses to management actions such as nutrient and sediment reductions, targeting restoration efforts in open water habitats, and evaluating overall ecosystem health. They will soon be used to measure progress towards plankton restoration goals. The differences and similarities in the ODU and Versar data evident in the Split Sample Study results were discussed at length by the monitoring program staffs, in their efforts to select a common method. The major issues that were debated are summarized in the following discussion and in Appendix F: "Tracking Sheet for Reviews of the April 2000 Draft Report on the Mesozooplankton Split Sample Study."

#### What are Mesozooplankton Taxa?

The question of whether or not the taxa undercounted in the CVS method were truly "mesozooplankton" was discussed throughout the split sample study. There was disagreement

on whether the CBP mesozooplankton monitoring programs should be counting a) organisms retained on a 200 micron mesh sieve in the laboratory, or b) organisms belonging to specific taxonomic groups and/or trophic levels that are retained in the monitoring programs' 202 micron mesh plankton nets in the field. A literature check indicates the latter is the preferred definition of mesozooplankton. Plankton categories have been proposed and refined for over a century, and the categories, or "functional groups," defined by Sieburth et al. (1978) are now widely accepted (Harris et al. 2000). Mesozooplankton are identified on the basis of taxonomy and trophic level, and are comprised mainly of copepod adults and copepodites in ocean settings but include cladocera, ostracods, and meroplankton larvae in estuarine waters (e.g. Seiburth et al. 1978, Harris et al. 2000, Day et al. 1989). Zooplankton as a whole span a wide size spectrum (six orders of magnitude) which necessitates grouping them into size fractions that can be effectively collected. The upper and lower limits chosen for each size fraction were selected so that they encompass the bulk of an individual zooplankton category (Sieburth et al. 1978). Since nets were - and still are - the primary means of collected zooplankton greater than 20 micron, this meant that plankton nets with mesh openings equal to the lower size limit should collect the bulk of an individual zooplankton category when towed correctly in the water. A size range of 200 micron - 20 mm (body length) was selected for the mesozooplankton even though immature individuals of some species are smaller than 200 microns and hence not adequately sampled by a 200 micron mesh plankton net. A brief overview of the five zooplankton categories and size ranges is given in Appendix E, and discussed in more detail in Sieburth et al (1978) and Harris et al. (2000).

Counts of certain zooplankton commonly caught in the plankton net tows were not used in the split sample study for various reasons:

- Large-sized copepod nauplii and rotifers: Versar and ODU submit mesozooplankton data sets to the CBP Data Center that include counts of large-sized copepod nauplii and rotifers which are technically microzooplankton (Appendix E). The monitoring program principal investigators discussed taking these microzooplankton counts out of the data sets submitted to the CBP Data Center in 1995 but chose to leave them in. It was thought that these counts of nauplii captured in a 200 u mesh plankton net tow may some day provide useful information about the proportion of larger copepod nauplii in the population. These microzooplankton counts are not used in calculations of bay-wide indicators, and they were not analyzed in the mesozooplankton split sample study.
- Fish eggs and larvae: ODU includes counts of fish eggs and larvae in data sets submitted to the CBP Data Center while Versar, Inc. does not. Versar's chief reason for excluding counts of these mesozooplankton taxa is that the staff believe the plankton nets currently used in the Maryland program do not adequately sample fish eggs and larvae. These counts were not analyzed in the mesozooplankton split sample study.

Counts of all other mesozooplankton taxa, even those with body lengths approaching 200 microns (e.g. early copepodite life stages, immature cladocerans, *Bosmina*, small ostracods, small meroplankton larvae), were analyzed in the split sample study.

The effects of net clogging and extrusion on the taxonomic composition of the mesozooplankton samples were discussed several times during the split sample study. The limitations of using a 202 micron mesh plankton net *in the field* to collect mesozooplankton taxa are recognized by both the Maryland and Virginia laboratories, and were a factor in their original choices of plankton net and sampling protocols. An unknown percentage of mesozooplankton taxa with

lengths and/or widths less than 200 microns are probably extruded from both the Maryland and Virginia plankton nets during towing. On the other hand, the plankton nets are clogged by detritus and phytoplankton during towing which somewhat counters the extrusion losses. Once concentrated in the bottle at the cod-end of the plankton net, the Maryland mesozooplankton samples are further concentrated with a 110 micron sieve before they are rinsed into sample jars and preserved while the Virginia mesozooplankton samples are simply washed into a 1-liter sample container and preserved. The Maryland ship-board sieving step is supported in zooplankton methodology manuals (e.g. Edmondson and Winberg 1971, Harris et al. 2000) but there is a risk that some mesozooplankton individuals could be extruded though the sieve. Possible losses during plankton tows and ship-board sieving would not affect the split sample results of this study.

# Counts from "Old" versus "New" CVS Methods

Count comparisons of the "old" and "new" CVS method used by ODU demonstrate that the "new" method counts for total mesozooplankton were approximately 1.50 times greater, or 50% larger, than the "old" method counts in the twenty-one, Round 2 split samples (Table 10). Thus, the "old" CVS method appears to undercount total mesozooplankton abundances. This study result is supported by a recent analysis of the 1985 - 1998 monitoring data which found that Versar and ODU total mesozooplankton counts for two adjacent stations in the Chesapeake Bay mainstem were significantly different (C. Buchanan, unpublished).<sup>3</sup> The median abundance was 2.42 times higher in Versar samples collected at Maryland station CB5.2 as compared to ODU samples collected at Virginia station CB6.1 and counted with the "old" CVS method. Together, the split sample and field results indicate that the pre-1999 mesozooplankton monitoring results in the Virginian Chesapeake Bay are undercounted. Further examination of Table 8 indicates that copepod and cladoceran counts gained the most when the method was changed while total counts for the miscellaneous group did not change significantly. Several individual taxa showed no significant differences on average between the "old" and "new" CVS method counts: adult Eurytemora affinis (frequent common calanoid copepod species in tidal freshwaters), adult Acartia spp. (dominant calanoid copepod genus in mesohaline/polyhaline salinities), Podonidae (mesohaline/polyhaline cladoceran family), harpacticoid copepods, and barnacle cypris and nauplii life-stages (meroplankton). If the "new" CVS method is instituted at ODU, these five taxa could possibly be used for long-term trends, thereby maintaining some backward comparability in Virginia. Only one of them proved to be directly comparable to Versar taxa counts, however.

<u>Versar vs ODU Taxa Counts</u> Count comparisons of all Round 2 split samples indicate that Versar's Stempel pipette counts for total mesozooplankton were still higher than ODU's "new" CVS method counts, despite increases in the ODU counts after adding the 64 micron sieve. The pipette method counts were on average 2.05 times greater than the CVS method counts. This

<sup>&</sup>lt;sup>3</sup> To reduce biases introduced by salinity-sensitive species, only 1985 - 1998 data points associated with salinities normally experienced by both stations (14.3 - 21.5 ppt) were used. The Versar median abundance was  $7,639/\text{m}^3$  (n=117) and the ODU median abundance was  $3,147.8/\text{m}^3$  (n=126). The Mann-Whitney test indicates the medians are significantly different (z = 2.6859, p<0.01). This degree of difference was not found between adjacent Maryland stations in the mesohaline waters (i.e. CB5.2 and CB4.3C) or adjacent Virginia stations (i.e. CB6.1 and CB6.4) in mesohaline/polyhaline waters.

translates to an *average* percent difference<sup>4</sup> of +42.9%, and a % difference for the *pooled* data (the sum of all Versar counts compared to the sum of all ODU counts) equal to +69% (Figure 5). Stempel pipette counts for all Round 2 counts of the three major mesozooplankton taxonomic groupings (copepods, cladocera, miscellaneous) and the dominant copepod order (calanoids) were also higher than the "new" CVS method counts (Figures 6, 7). The twenty-one Versar and ODU split sample counts were roughly the same for the less common cyclopoid copepods (-2.8%), and "new" CVS counts for the rarer harpacticoid copepods were larger than the pipette counts (Figure 6). This latter result is unexpected because split counts for copepods as a whole were higher with the pipette method.

When Stempel pipette counts are compared to "new" CVS method counts on a taxa by taxa basis, it appears as if four abundant taxa are primarily responsible for the observed differences between the Versar and ODU total counts: copepodite *Eurytemora affinis*, copepodite *Acartia tonsa*, barnacle nauplii and chydorids. Versar counts of copepodite *Eurytemora affinis*, the most abundant taxa in this split sample study, were 3.78x greater than the ODU counts (Table 8). Copepodite *Acartia tonsa*, barnacle nauplii, and chydorids were, respectively, 2.25x, 2.27x, and 4.76x more abundant in the Versar counts. The differences in total mesozooplankton counts caused by the higher Versar taxa counts are partially countered by taxa differences in the opposite direction caused by a few higher ODU taxa counts. These latter differences are unusual because they occur within taxonomic groupings where the Versar count is higher. For example, ODU counts for cyclopoid copepods and for "other calanoids" (excludes *Acartia* and *Eurytemora*) were higher than Versar's, yet ODU *total* copepod counts were lower than Versar's (Table 8). The countervailing differences in some taxa indicate laboratory inconsistencies in taxonomic identification are still occurring that need to be found and resolved.

Further comparisons of the split samples suggest Versar Stempel pipette counts and ODU "new" CVS method counts for four relatively abundant taxa might be directly comparable: adult *Eurytemora affinis*, adult *Acartia tonsa*, Podonidae, and *Bosmina* (Table 8). If the Chesapeake Bay Program decides to maintain two different mesozooplankton counting protocols for the sake of backward compatibility with the pre-1999 data (i.e. it accepts ODU counts produced by the "new" CVS method and continues to accept Versar counts produced by the Stempel pipette method), then these four taxa have the greatest potential for being directly comparable in post-1998 Virginia and Maryland monitoring data. Their direct comparability would need to be confirmed with additional split samples. While these four taxa are important constituents of the zooplankton community and seasonally abundant, bay-wide evaluations of zooplankton community health that are based solely on these four species will not be adequate for the Chesapeake Bay Program.

The possibility using the split sample results to develop correction factors to adjust mesozooplankton counts in the pre-1999 CBP monitoring data was discussed during the course

<sup>&</sup>lt;sup>4</sup> Percent difference is the difference of the Versar and ODU counts for a split sample divided by their mean, then multiplied by 100. Positive values indicate Versar counts are higher; negative values indicate ODU counts are higher. Values greater than 120% can be considered significantly different (p<0.05). The average % difference is the average of the % differences for several of samples. The % difference of the pooled sample data is obtained by calculating % difference on the sum of all Versar counts and the sum of all ODU counts being compared. By summing the Versar and ODU counts, arbitrary biases introduced by small raw counts in a few of the split samples is minimized.

of the study. Correction factors were calculated for abundant taxa in the "Last Ten" split samples (Table 8), however, sample by sample comparisons of taxa differences suggest that the variability experienced in taxa life-stage sizes will result in unstable correction factors and the attempt to develop the factors was discarded. Three taxa might not need correction factors to be directly comparable in the pre-1999 CBP monitoring data: adult *Eurytemora affinis*, adult *Acartia tonsa*, and Podonidae. All three appear to be minimally affected by the "old" to "new" CVS method change (see above), and the Split Sample Study indicates their "new" CVS method counts and Stempel pipette counts are directly comparable. Analysis of the monitoring data warns against this conclusion for *Acartia*, however (Figure 8). Actual Stempel pipette counts of *Acartia* in the Maryland samples were 4.3 times greater than the "old" CVS method counts in the ODU samples over the 14 - 21 ppt salinity range. On the other hand, the monitoring data suggest that Versar and ODU field counts of adult *Eurytemora affinis* might be comparable (Figure 9).

# Does the CVS method undercount mesozooplankton?

The "old" CVS method very clearly undercounted mesozooplankton. Comparisons of "new" and "old" CVS method counts show that total counts and most taxa counts increased significantly when a smaller sieve was added. Hence, most counts obtained with the "old" CVS method (i.e. the 1985-1998 Virginia monitoring data) are undercounted. The lower split sample counts obtained with the "old" CVS method appear to be due primarily to sieving losses through the bottom 200 micron sieve. While the CVS method as originally described in Alden et al. (1982) employed four large-mesh sieves in combination with a 150 micron and a 75 micron mesh sieve, the "old" CVS method used by the Virginia mesozooplankton monitoring program since its inception employed five large-mesh sieves (2000, 850,600, 300, 200 microns) and no small-mesh sieves (i.e. <200 microns).

The significantly lower counts produced by the "new" CVS method in Round 2 of the Split Sample Study indicate one or both methods are not producing counts representative of actual mesozooplankton abundances in the field. Is the Stempel pipette method biasing counts above actual sample levels, or the "new" CVS method biasing counts below actual sample levels, or both? The possibility of bias in the Stempel pipette counts caused by clumping was tested for several years by Versar, and did not appear to be occurring (W.Burton, personal communication). Also, replicate sample counts perform regularly by Versar indicate good repeatability (W. Burton, personal communication). These QA/QC data could be further analyzed if needed to check the accuracy of the existing Versar counts. Information from several zooplankton methodology manuals suggest that aspects of the "new" CVS method could be causing it to undercount the ODU samples. First, several distinct taxa with significantly lower ODU split sample counts are large but also narrow, e.g. Eurytemora affinis and Acartia tonsa copepodites. A review of the lengths and widths of commonly found mesozooplankton taxa in Chesapeake Bay (Table 11) suggests many immature copepods could be extruded head-first through the bottom 64 micron mesh sieve of the "new" CVS method as the sieves are shaken during the sieving process. Second, several methodology manuals suggest animals stick to the walls of sample splitters and a percentage could be lost during the CVS method splitting steps with the Folsom splitter (e.g. Edmondson and Winberg, 1971, pg 130; APHA, 1995). Finally, use of an unleveled Folsom splitter will produce biases in the subsamples which increase with repeated splitting (APHA, 1995). One or more of these causes of bias could be responsible for the lower ODU counts, but further tests would be need to done to determine if they are in fact occurring.

# Versar-ODU Differences in Taxa Richness and Diversity

The modified mesozooplankton counting methods do not produce comparable taxa richness measures (Figure 10). While taxa richness increased when a sieving step (850 microns) was added to the Versar laboratory protocol and counts for larger, rarer taxa were reinstated, the Split Sample Study shows that other issues still need to be resolved before taxa richness or taxa diversity indices can be used bay-wide. First, level of taxonomic identification is not consistent between the states. Side-by-side count comparisons by program staff at the March 1999 meeting served to move the two laboratories closer to a common level of taxonomy, but the species lists are not identical yet. The problem was overcome in the split sample study by "lumping" species counts into higher taxonomic categories, but this is not desirable long-term solution. Second, fewer species were observed in the ODU counts (Figure 10). While the CVS sieving steps and the addition of an 850 micron sieving step to the Versar method both help to bring forward largesized, rare species for counting, other aspects of the CVS method are making the number of observed taxa in the ODU splits lower than those in the Versar splits. The second issue, in combination with the lower ODU total counts, brings into question the usefulness of Margalef's Diversity Index as a bay-wide indicator of zooplankton community health at the present time. Taxa richness (number of observed taxa) is a variable in the index numerator and total abundance (number of organisms per sample) is a variable in the denominator. When richness is divided by abundance, as in Margalef's Diversity Index, the resulting proportion does not reflect the lower taxa richness and lower total abundance of the CVS method counts. Thus, the Virginia and Maryland diversity indexes were approximately the same (Figure 10). The Shannon-Wiener, Pielou, and Simpson indices of diversity would be similarly affected because they also rely on measures of species proportional abundance.

#### Sample Variances

A higher level of sample variance was observed in the ODU counts (Figures 2, 4). This reflects Versar's choice of a ±20% error level and ODU's choice of a 35% error level (see Introduction, Appendix A). Versar achieves its lower error level by producing relatively large raw counts (Table 12). The 20% and 35% error levels are for total mesozooplankton counts, and error levels for individual taxa are usually much higher. This was evident in the split sample results for rarer taxa which typically had very high % differences. The higher sample variance and subsequently lower estimates of precision for the ODU sample counts make it more difficult to identify significant trends in the Virginia data as compared to the Maryland data. These difficulties are overcome by time in long-term data sets. However, the CBP in its search for ecosystem responses to nutrient reductions is very interested in year to year trend changes in the monitoring parameters. Both programs should probably take this management need into consideration when future approaches and levels of effort are discussed.

# Next Steps

Monitoring program representatives did not reach a consensus on which method should be adopted by both laboratories after they reviewed the Split Sample Study results. To help them decide, they agreed to perform additional split sample comparisons to determine if laboratory differences were due to method bias or technician bias, or both. If the results confirm this report's conclusions and bias is shown to be method dependent, the representatives agreed that one method should be selected for both laboratories. The method that yields comparable results, and the best precision and bias will be selected.

#### **Conclusions**

# 1. Inter-laboratory split sample comparisons between ODU and Versar indicate that the laboratories do not produce comparable abundance data for most species. There were:

- Persistent differences in level-of-taxonomy for some taxa groups
- Persistent differences in the taxonomic identifications for at least chydorid cladocerans, "other" cladocerans, ostracods, and several copepod taxa
- Significantly higher Versar counts for "total mesozooplankton"
- Significantly higher Versar counts for "total copepod"

Within the copepod group, Versar counted significantly higher "total calanoid copepods," and calanoid copepodite life stages (i.e. *Acartia*, *Eurytemora*) while ODU counted significantly higher "total harpacticoid copepods" and the laboratories produced roughly comparable counts for "total cyclopoid copepod."

- Significantly higher Versar counts for "total cladocerans"
- Slightly higher Versar counts for "total miscellaneous" (includes ostracods, polychaetes larvae, immature barnacles, and other meroplankton larvae)
- Greater taxa richness in the Versar samples
- Lower coefficients of variance (CV) in the Versar split samples than in ODU samples In general, mesozooplankton with the smallest body sizes and/or the narrowest body shapes appear to be most affected by the CVS counting method, i.e. ODU count differences with Versar are frequently greatest in these taxa. Calanoid copepodites may be especially undercounted by the ODU method.

# 2. Split sample comparisons between counts produced with the "new" Versar method and the "new" ODU method identified areas of uncertainty and areas of agreement/improvement:

- Possible taxonomic differences between counters within at least one of the laboratories during 1998
- Counts of four taxa are in general agreement a) between laboratories, and b) between "old" and "new" ODU methods.

Counts for these four taxa could possible be used for long-term trends, thereby maintaining some backward comparability in Virginia. The four taxa showed no significant differences *on average* between the "old" and "new" ODU counts, although their sample variances were at times large. ODU counts of these taxa were also generally comparable to Versar counts. These taxa are: <u>adult Eurytemora affinis</u> (frequent common calanoid copepod species in tidal freshwaters), <u>adult Acartia spp.</u> (dominant calanoid copepod genus in mesohaline/polyhaline salinities), Podonidae (mesohaline/polyhaline cladoceran family), and possibly *Bosmina longirostris* (seasonally dominant cladoceran in freshwater).

- Improvement in the quality of Versar and ODU taxa counts as a result of site visits, side-byside taxonomic comparisons, and the split sample study
- 3. Differences between laboratories may be due to method bias or technician bias, or both. Further work is needed to determine bias. If bias is shown to be method dependent, one method will be selected for both laboratories. The method that yields comparable results, and the best precision and bias will be selected.

Specific recommendations include:

• Determine which method is truly biased, i.e., is the CVS method underestimating counts or is the modified Stempel pipette method overestimating?

Check if CVS method is biased low due to sieving loss. Reanalyze one sample multiple times. Diminishing recoveries of species abundances will indicate loss. Check if CVS method bias is due to Folsom splitter. Follow Standard Methods 19<sup>th</sup> edition procedure to verify that the splitter is unbiased and to determine the sampling error introduced by using it.

Analyze a sample of known species identities and abundances with the CVS and Stempel pipettes each method. This comparison should be done within ODU and between ODU and Versar. (Custom made sample)

Check the Stemple pipette method subsampling and sorting bias using procedure in section 2.1.8 of the IPB Handbook (Edmondson and Winberg 1971). Both Versar and ODU need to do this.

- Determine technician bias by comparing results from the Stempel pipette method performed by both ODU and Versar.
- 4. Quality assurance counts within each laboratory and between laboratories should be rigorously maintained, documented, and periodically reviewed to ensure comparable, high quality mesozooplankton counts. Quality assurance procedures should be maintained in each laboratory to ensure adequate taxonomic training of new technical staff. Quality assurance (repeated) counts for each laboratory should be regularly submitted to the states, the Chesapeake Bay Program or their designees for independent analysis. Regular site visits between the two states' technical staffs should be carried out to ensure comparable interstate taxonomy. A split sample study should be done annually for at least the next few years to ensure interstate count comparability.
- 5. Both laboratories should work from an identical taxon list, to the same level of taxonomy, and they should enumerate the same life stages. A record of the mesozooplankton taxa identified in the CBP zooplankton monitoring program should be maintained in both laboratories (e.g. a type specimen collection, a photographic record). Laboratory differences in taxonomic identifications can be reconciled during side-by-side comparisons and through the assembly of a photographic or type specimen collection for Chesapeake Bay mesozooplankton. The goal would be to standardize the level of taxonomy and avoid discrepancies in taxonomic identification between laboratories.

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Figure 1. Comparison of Total Mesozooplankton Counts in Round 2 First Ten Split Samples. The average % difference between the Versar and ODU total mesozooplankton counts for these samples was +13%. The % difference of the pooled sample data is +69.0%. In most cases, the individual sample % differences were greater than +20%, suggesting a high degree of variability in the counts from one or both laboratories. Note: the RET3.1 May sample was counted twice by ODU. Details: Percent (%) difference is the difference of the Versar and ODU counts for a split sample, divided by their mean. Positive values indicate the Versar count was highest. Negative values indicate the ODU count was highest. Values higher than +20% or lower than -20% can be considered significantly different (p<0.05). The average % difference is the average of all the individual sample % differences. The % difference of the pooled sample data is obtained by calculating % difference on the sum of all Versar counts and the sum of all ODU counts. By summing the Versar and ODU counts, arbitrary biases introduced by small raw counts in a few of the split samples is minimized.

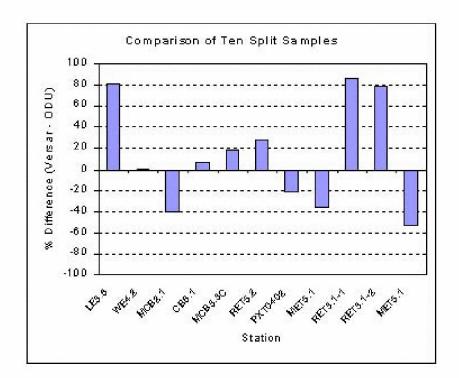


Figure 2. Plot of Versar Coefficient of Variation vs ODU Coefficient of Variation in Round 2 First Ten Split Samples (Elgin Perry 15:11 Thursday, January 28, 1999). VCV = Versar Coefficient of Variation, OCV = ODU Coefficient of Variation. Legend: A = 1 obs, B = 2 obs, etc. N = 71 (i.e. splits samples where taxon counts are available from both laboratories.) The results indicate the ODU split samples have a higher coefficient of variation than the Versar split samples (i.e. they fall below the VCV = OCV diagonal line).

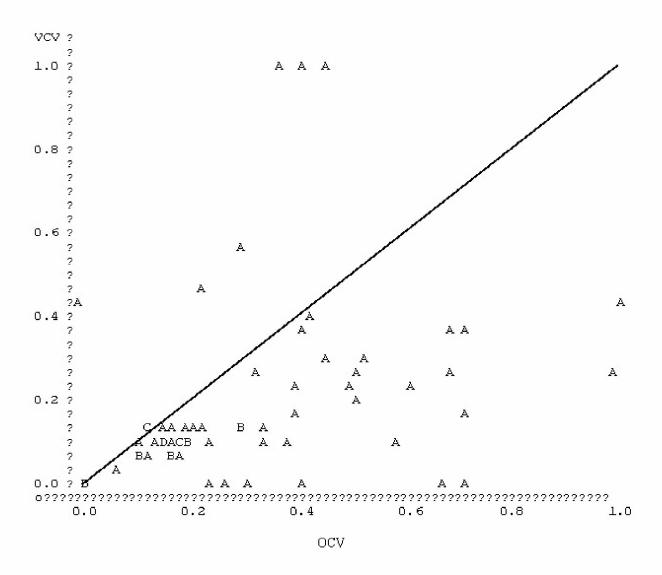


Figure 3. Percent (%) difference between ODU and Versar total mesozooplankton counts, by sample, for the Round 2 Last Ten split samples. (See Figure 1 caption for details.) The average % difference of the 10 samples is 74.9%. The % difference between the pooled ODU and pooled Versar counts is 67.2%.

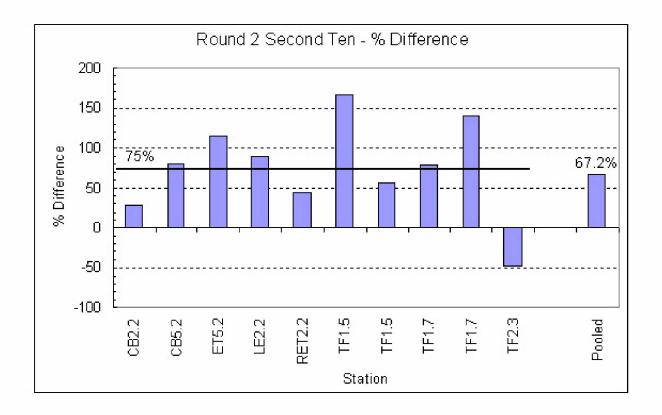


Figure 4. Plot of Versar Coefficient of Variation vs ODU Coefficient of Variation in Round 2 Second Ten Split Samples. N = 62 (i.e. splits samples where taxon counts are available from both laboratories.) The results indicate the ODU split samples have a higher coefficient of variation than the Versar split samples (i.e. they fall below the VCV=OCV diagonal line).

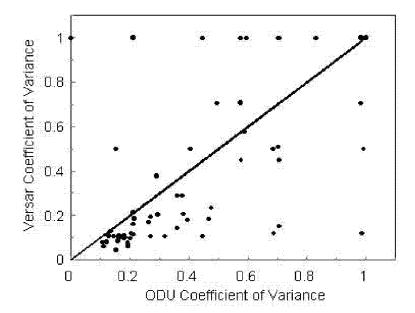


Figure 5. Percent (%) difference between ODU and Versar total mesozooplankton counts, by sample, for all Round 2 split samples (n = 21). (See Figure 1 caption for details.) There is a preponderance of positive % differences in the 21 counts of total mesozooplankton, indicating Versar counts are generally higher. The average % difference is +42.9%. The % difference of the pooled sample data is +69.0%. Note: the RET3.1 May sample was counted twice by ODU, so there are 21 counts for 20 split samples.

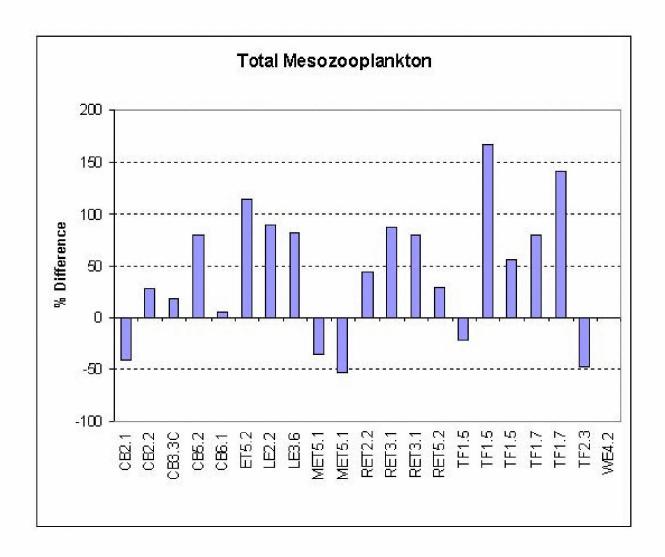


Figure 6. Percent (%) differences of pooled taxa data for Round 2 "First Ten" and "Second Ten" of the Split Sample Study. (See Figure 1 caption for details.) "Mesozooplankton" consists of all the mesozooplankton taxa (dark colored bars). "Miscellaneous" (primarily meroplankton larvae and ostracods), "cladocera" and "copepod" are the three major taxonomic groupings of mesozooplankton in estuaries (light colored bars). "Calanoid," "cyclopoid," and "harpacticoid" are three orders of copepod (white bars). The % difference for the "Miscellaneous" and "Cladocera" taxonomic groupings are only shown for the second ten split samples because taxonomic identification changes made by ODU and Versar after the first ten split samples affect the earlier results. Only the second ten split sample results for "Harpacticoid" are shown because counts of harpacticoids in the first ten were relatively small. Data for groups that are not know at this time to have taxonomic identification problems can be pooled for all Round 2 split samples, and their overall % differences are: total mesozooplankton, +69.0%; calanoid copepods, +65.1%; cyclopoid copepods, -2.8%; harpacticoid copepods, 45.0%. The most abundant copepod group in Chesapeake Bay is the calanoid copepod, and counts for this group tend to dominate the "copepod" results (see graph).

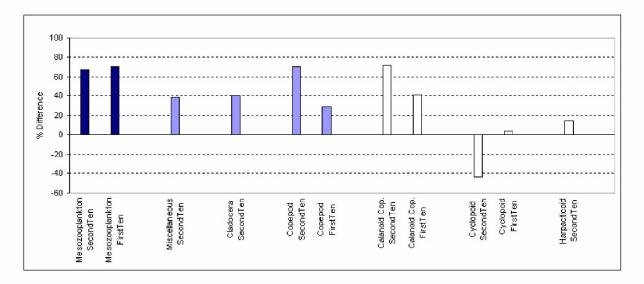


Figure 7. Percent (%) difference for ODU and Versar total copepod counts for all Round 2 split samples (see Figure 1 caption for details). The average % difference of the total copepod counts is +48.1%. The % difference of the pooled sample data is +59.0%.

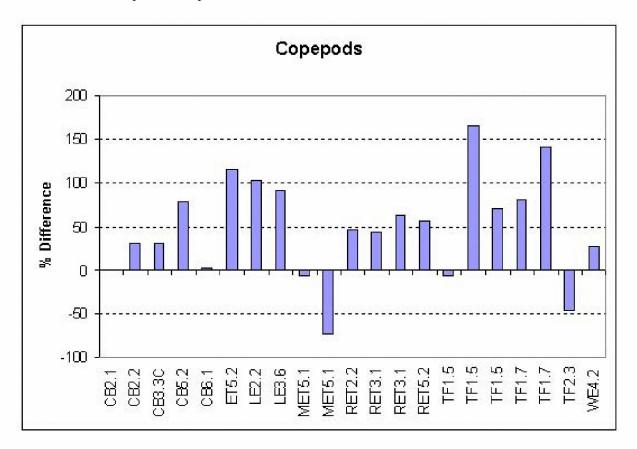
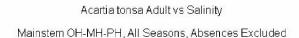


Figure 8. Comparison of Versar, Inc. and Old Dominion University (ODU) estimates of Acartia tonsa adult abundances in oligohaline, mesohaline and polyhaline salinities of the Chesapeake Bay mainstem versus salinity (all sample dates between August 1984 and December 1998). Absences, or zero values, are excluded. The graphs demonstrate the euryhaline nature of Acartia tonsa, i.e. salinity between approximately 5 and 32 ppt do not affect abundances. Secchi depths experienced in this salinity range overlapped strongly and are not a reason for the laboratory differences (i.e. secchi depths of 0.4 - 4.5 m were experienced in ODU data and secchi depths of 0.5 - 5.5 m were experienced in Versar data). Versar and ODU counts for adult Acartia tonsa were significantly different (p<0.01) at the adjacent CB5.2 (Maryland) and CB6.1 (Virginia) mainstem stations, the most comparable of the Maryland and Virginia mainstem stations. Versar's counts were approximately 4.33 times greater than ODU's (C. Buchanan, unpublished).



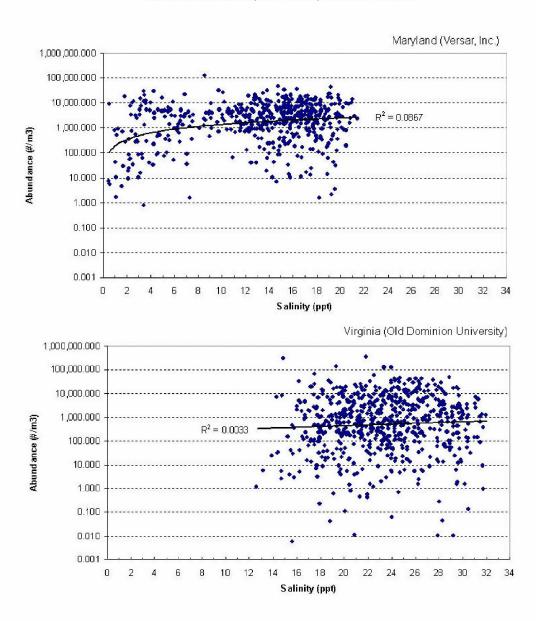


Figure 9. Adult Eurytemora affinis monitoring data from the Maryland and Virginia mainstem (1985 - 1998), for salinities that occur in both states (14 - 21 ppt). Light points and line: Virginia data; dark points and line: Maryland data. Although primarily an oligohaline/low mesohaline species, Eurytemora affinis is found in the Chesapeake Bay middle mainstem. The effect of salinity can be seen in the negative slopes of the regression lines. The Maryland and Virginia regressions are nearly identical, suggesting that Versar Stempel pipette counts and ODU "old" CVS method counts could be comparable.

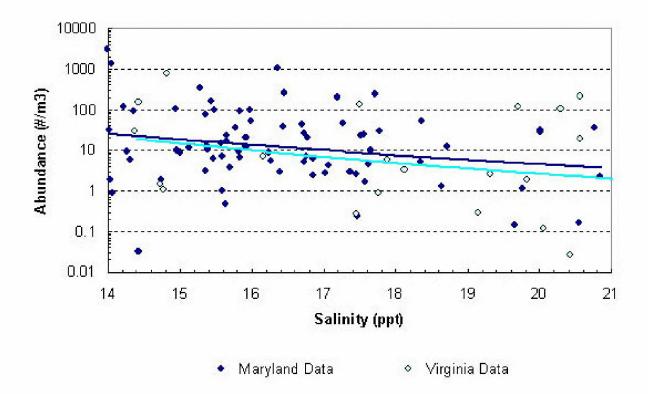


Figure 10. Total mesozooplankton abundance, species richness and Margalef Diversity Index for "Last Ten" split samples of Round 2. The % differences of the pooled data (see Figure 1 caption for details) are shown in the graphs.

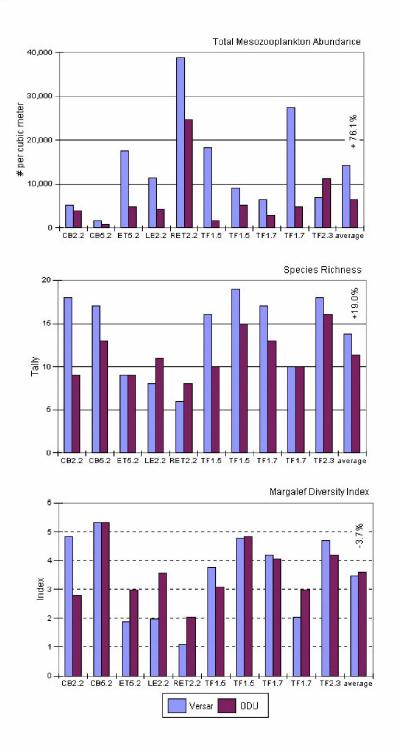


Table 1. Total, non-normalized mesozooplankton counts (i.e. total number per sample jar, estimated from raw subsample counts and total sample volume) for the side-by-side split sample comparisons done at ODU in early 1998. The high Versar count at PXT0402 (= TF1.5) is due to abundant *Bosmina longirostris*. The high Versar count at XDA1177 (= RET2.2) is due to abundant *Acartia tonsa* adults and copepodites.

Salinity	Station	Sample	Taxa	Old Versar	Old ODU	ODU count is this
		Date				% of the Versar coun
TF	PXT0402	n/a	TOTAL	7,346,400	65,664	0.89
он	XDA1177	n/a	TOTAL	1,301,082	154,967	11.91
он	MCB2.2	n/a	TOTAL	18,639	6,236	33.46
мн	MCB4.3C	n/a	TOTAL	264,000	164,460	62.30
мн	MLE2.2	n/a	TOTAL	32,550	1,632	5.01

Table 2. Mesozooplankton Summary Statistics Comparison, September 11-12, 1998, Using New Methods. Units are number per m³ (abundance) or ug Carbon per m³ (biomass), except for the diversity index.\*

Ctation	Data	Counting	TotMes Abundance	Calanoid Abundance	Cladoceran	Cyclopoid	Cal:Cla&Cyc	Ostracod Abundance	Polychaete Abundance
Station CB6.1	<b>Date</b> 5/18/98	Agency Versar	1.881.62	1,521.83	Abundance 173.81	Abundance 2.18	<b>Ratio</b> 8.65	23.29	4.16
CB6.1	5/18/98	ODU	715.18	560.17	1/3.81	0.00	5.13	0.00	0.00
CB0.1 CB7.3E	3/6/98	Versar	140.87	134.46	0.21	3.17	39.77	1.48	0.00
CB7.3E	3/6/98	ODU	7.90	2.66	2.33	0.09	1.11	0.00	0.42
CB7.3L CB7.4	4/8/98	Versar	5,031.44	2,301.99	62.16	1,026.67	2.11	6.02	50.13
CB7.4	4/8/98	ODU	10,186.69	6,182.64	800.80	1,334.67	2.90	0.00	369.60
LE3.6	6/1/98	Versar	756.33	724.41	7.19	0.00	100.71	16.44	0.00
LE3.6	6/1/98	ODU	604.40	427.54	119.03	0.00	3.59	0.00	0.00
MCB2.1	5/6/98	Versar	706.10	104.88	496.82	73.82	0.18	1.16	0.00
MCB2.1	5/6/98	ODU	686.48	33.49	589.00	51.17	0.05	0.00	0.00
MCB3.3C	5/5/98	Versar	6,271.03	4,850.43	0.01	8.46	572.27	3.05	384.62
MCB3.3C	5/5/98	ODU	3,457.09	3,227.08	6.30	1.56	410.40	0.00	0.00
MCB4.3C	4/7/98	Versar	39,776.83	39,425.37	0.00	25.86	1,524.45	12.93	90.52
MCB4.3C	4/7/98	ODU	14,643.10	12,424.83	971.03	11.03	12.65	0.00	0.00
MCB4.3C	6/2/98	Versar	2,316.99	2,272.88	1.63	0.00	1,391.00	16.34	3.27
MCB4.3C	6/2/98	ODU	1,354.94	1,326.08	1.08	0.03	1,193.49	0.00	0.00
MET5.1	4/7/98	Versar	10,753.29	3,295.92	7,294.01	86.74	0.45	25.51	0.00
MET5.1	4/7/98	ODU	3,466.94	230.82	3,157.76	23.67	0.07	32.65	0.00
MET5.1	5/5/98	Versar	38,800.20	1,396.10	24,775.72	12,548.70	0.04	32.47	0.00
MET5.1	5/5/98	ODU	60,614.81	531.95	45,958.18	13,651.43	0.01	33.25	0.00
MLE2.2	5/4/98	Versar	17,561.10	14,890.93	291.67	20.83	47.65	520.83	62.50
MLE2.2	5/4/98	ODU	23,327.83	18,476.17	551.17	0.00	33.52	171.00	0.00
MWT5.1	6/3/98	Versar	16,926.86	15,235.29	51.47	12.26	239.08	39.22	19.61
MWT5.1	6/3/98	ODU	4,573.33	3,917.65	49.41	28.33	50.39	3.14	0.00
PXT0402	4/13/98	Versar	31,186.12	29,364.16	1,345.12	175.45	19.31	184.97	23.12
PXT0402	4/13/98	ODU	19,167.40	16,369.48	2,146.24	177.57	7.04	252.02	0.00
PXT0402	6/8/98	Versar	10,905.23	4,809.06	5,971.45	91.76	0.79	0.00	0.00
PXT0402	6/8/98	ODU	10,782.85	5,378.58	4,933.01	339.68	1.02	75.60	0.00
RET3.1	5/6/98	Versar	127,047.47	6,875.27	116,811.08	3,175.43	0.06	43.46	0.36
RET3.1	5/6/98	ODU	8,286.80	4,195.89	3,993.02	29.76	1.04	0.00	0.00
RET4.3	6/10/98	Versar	390.99	289.79	10.42	3.48	20.85	7.64	0.00
RET4.3	6/10/98	ODU	193.05	104.93	13.34	1.25	7.19	1.11	0.00
RET5.2	4/22/98	Versar	11,853.31	957.51	10,346.98	520.73	0.09	12.44	4.15
RET5.2	4/22/98	ODU	8,091.15	854.38	6,774.47	445.85	0.12	0.00	0.00
SBE5	5/14/98	Versar	1,692.85	1,630.41	1.40	2.37	432.59	2.37	0.21
SBE5	5/14/98	ODU	1,183.39	1,101.63	0.00	0.00	110,163.16	0.00	0.00
TF3.3	6/10/98	Versar	24,345.22	13,766.09	6,922.53	3,510.89	1.32	0.30	0.00
TF3.3	6/10/98	ODU	26,136.32	1,824.79	20,035.11	3,842.14	0.08	0.00	0.00
TF4.2	4/10/98	Versar	994.55	943.89	26.19	11.08	25.32	0.59	1.01
TF4.2	4/10/98	ODU	921.35	828.85	49.56	0.00	16.72	0.00	10.32
TF5.5	5/20/98	Versar	2,116.40	1,747.52	225.41	140.12	4.78	0.00	0.00
TF5.5	5/20/98	ODU	4,921.85	2,321.76	305.92	2.96	7.52	18.35	0.00
WE4.2	4/6/98	Versar	862.10	567.66	49.27	0.51	11.40	8.64	16.25
WE4.2	4/6/98	ODU	1,224.18	601.06	66.97	0.00	8.98	0.00	22.11
XDE5339	4/13/98	Versar	5,406.42	3,494.74	5.26	15.79	166.00	5.26	31.58
XDE5339	4/13/98	ODU	1,532.63	957.90	16.00	0.21	59.09	0.00	0.00
XEA6596	6/1/98	Versar	5,678.40	2,493.87	1,086.31	2,098.21	0.78	0.00	0.00
XEA6596	6/1/98	ODU	311.79	120.00	75.36	115.36	0.63	0.00	0.00

Table 2 (Cont.)

Mesozp	Copepod	Adult Copepod	Copepodite	Total Copepod	Adult Copepod	Copepodite	Margalef	Station	Date
Biomass	Biomass	Biomass	Biomass	Abundance	Abundance	Abundance	Diversity		
3,801.37	3,620.65	2,874.71	745.95	1,524.01	900.31	623.70	2.75	CB6.1	5/18/98
1,819.95	1,756.97	1,736.10	20.88	560.17	542.87	17.30	1.75	CB6.1	5/18/98
294.03	290.31	204.18	86.13	137.63	65.78	71.85	8.38	CB7.3E	3/6/98
9.43	7.48	6.72	0.76	2.75	2.11	0.63	11.14	CB7.3E	3/6/98
5,964.36	4,632.30	2,163.16	2,469.14	3,328.66	1,463.81	1,864.85	4.86	CB7.4	4/8/98
17,651.32	15,428.83	11,800.40	3,628.43	7,517.31	4,827.23	2,690.07	3.74	CB7.4	4/8/98
1,703.25	1,689.25	1,314.43	374.83	724.41	411.02	313.40	2.08	LE3.6	6/1/98
1,463.17	1,354.41	1,335.53	18.88	432.80	417.02	15.78	4.31	LE3.6	6/1/98
645.44	306.02	99.86	206.16	190.28	42.09	148.19	8.78	MCB2.1	5/6/98
609.31	234.95	215.12	19.83	93.03	78.18	14.85	7.05	MCB2.1	5/6/98
10,676.90	8,979.29	4,969.69	4,009.60	4,860.69	1,649.45	3,211.23	3.16	MCB3.3C	5/5/98
9,438.87	9,265.73	8,980.14	285.59	3,228.65	3,002.76	225.89	2.54	MCB3.3C	5/5/98
70,310.73	69,771.96	29,064.36	40,707.59	39,451.23	10,917.28	28,533.94	4.13	MCB4.3C	4/7/98
24,780.44	24,066.38	12,888.82	11,177.56	12,435.86	4,889.66	7,546.21	2.16	MCB4.3C	4/7/98
5,171.66	5,141.37	3,871.11	1,270.26	2,272.88	1,210.78	1,062.09	1.78	MCB4.3C	6/2/98
4,051.83	4,031.57	3,908.03	123.55	1,326.11	1,222.81	103.30	2.55	MCB4.3C	6/2/98
9,507.47	5,128.50	596.01	4,532.49	3,408.16	234.69	3,173.47	3.97	MET5.1	4/7/98
2,496.54	605.61	501.57	104.04	263.47	193.27	70.20	3.39	MET5.1	4/7/98
38,732.72	23,329.41	9,824.29	13,505.12	13,944.81	3,754.06	10,190.75	4.79	MET5.1	5/5/98
60,720.45	32,169.41	27,055.53	5,113.88	14,183.64	10,327.01	3,856.62	4.39	MET5.1	5/5/98
33,847.95	31,875.71	22,414.96	9,460.75	14,911.76	7,015.93	7,895.83	2.12	MLE2.2	5/4/98
61,181.34	57,707.33	56,890.86	816.47	18,476.17	17,793.50	682.67	2.06	MLE2.2	5/4/98
25,560.10	24,296.68	9,656.71	14,639.98	15,247.55	3,019.61	12,227.94	2.60	MWT5.1	6/3/98
12,445.68	11,977.52	11,594.57	382.96	3,946.08	3,625.88	320.20	3.83	MWT5.1	6/3/98
54,221.89	53,305.78	24,453.30	28,852.49	29,562.74	9,447.13	20,115.61	3.78	PXT0402	4/13/98
39,044.58	37,623.28	30,430.18	7,193.10	16,621.04	11,767.40	4,853.64	3.27	PXT0402	4/13/98
12,737.80	9,210.77	4,901.24	4,309.53	4,913.76	1,900.25	3,013.52	3.96	PXT0402	6/8/98
18,147.48	15,099.66	15,099.66	0.00	5,718.25	5,718.25	0.00	3.22	PXT0402	6/8/98
89,740.31	19,388.48	11,814.69	7,573.79	10,073.88	4,542.95	5,530.93	5.68	RET3.1	5/6/98
16,428.18	14,009.31	14,009.31	0.00	4,255.34	4,255.34	0.00	2.30	RET3.1	5/6/98
2,165.51	885.96	855.72	30.25	293.67	268.65	25.02	9.26	RET4.3	6/10/98
1,457.11	131.44	5.91	125.53	106.74	2.09	104.66	6.56	RET4.3	6/10/98
8,926.84	2,476.01	891.76	1,584.25	1,482.38	342.49	1,139.90	6.63	RET5.2	4/22/98
6,840.14	2,692.20	1.662.14	1,030.06	1,300.23	605.18	695.05	3.33	RET5.2	4/22/98
4,829.33	4,061.25	3.358.52	702.73	1,651.77	1,070.34	581.43	7.43	SBE5	5/14/98
5,600.96	3,314.11	3,129.05	185.06	1,130.11	975.38	154.73	3.58	SBE5	5/14/98
33,628.84	29,233.98	13,154.52	16,079.46	17,357.38	5,614.74	11,742.64	6.38	TF3.3	6/10/98
32,826.01	13,400.32	10,695.98	2,704.34	5,941.37			2.49	TF3.3	6/10/98
1,837.38	1,819.58	1,005.59	814.00	957.99	388.84	•	7.67	TF4.2	4/10/98
3,599.31	2,231.95	1,840.75	391.19	834.01	563.07		6.75	TF4.2	4/10/98
3,670.14	3,535.62	1,843.28	1,692.35	1,890.80	707.86		6.92	TF5.5	5/20/98
6,207.63	3,923.72	836.51	3,087.21	2,336.96	253.82	*	3.79	TF5.5	5/20/98
1,837.18	1,618.57	1,489.52	129.05	568.17	464.04		5.11	WE4.2	4/6/98
2,398.01	1,940.98	1,409.32							4/6/98
7,315.57	5,755.17	2,384.06	0.00 3,371.11	601.06 3,521.05	601.06 763.16		3.24 3.22	WE4.2 XDE5339	4/13/98
	2.730.48								
3,224.48 9,657.26	6,655.26	2,519.89 2,234.76	210.59 4,420.50	961.47 4,592.09	789.47 922.62		2.83 2.66	XDE5339 XEA6596	4/13/98 6/1/98
	·	*				·			
783.66	628.79	627.52	1.26	236.31	235.36	0.95	4.01	XEA6596	6/1/98

Table 3. Round 1 split sample results as percent differences for specific taxon groups (%). Percent difference was calculated as follows: (Versar # - ODU #)/((Versar # + ODU #)/2) \* 100. Positive numbers indicate Versar's counts are higher, negative numbers indicate ODU's counts are higher.

STATION	DATE	TOTAL MESOZOOP ABUNDANCE	CALANO ID COPEPOD ABUNDANCE	CLADOCERAN ABUNDANCE	CYCLO POID COPEPOD ABUNDANCE	OSTRACOD ABUNDANCE	POLYCHEATE ABUNDANCE	COPEPOD ABUNDANCE	COPEPOD ABUNDANCE - ADULTS	COPEPOD ABUNDANCE - COPEPODITES	MARGAELF DIVERSITY
CB6.1	5/18/98	89.84	92.38	45.74	200.00	200.00	200.00	92.49	49.54	189.21	44.27
CB7.3E	3/6/98	178.75	192.23	-166.72	189.55	200.00	200.00	192.17	187.55	196.50	-28.31
CB7.3L	4/8/98	-67.75	-91.47	-171.19	-26.09	200.00	-152.23	-77.24	-106.93	-36.23	26.05
LE3.6	6/1/98	22.33	51.54	-177.21	20.07	200.00		50.40	-1.45	180.82	-69.71
MCB2.1	5/6/98	2.82	103.19	-16.98	36.24	200.00		68.66	-60.02	163.57	21.79
MCB3.3C	5/5/98	57.85	40.19	-199.24	137.65	200.00	200.00	40.35	-58.18	173.71	21.64
MCB4.3C	4/7/98	92.37	104.15	-200.00	80.38	200.00	200.00	104.13	76.27	116.34	62.62
мсв4.3С	6/2/98	52.40	52.62	41.00	-200.00	200.00	200.00	52.61	-0.99	164.54	-35.55
MET5.1	4/7/98	102.48	173.82	79.15	114.23	-24.56		171.30	19.36	191.34	15.74
MET5.1	5/5/98	-43.89	89.64	-59.89	-8.42	-2.37		-1.70	-93.36	90.18	8.78
MLE2.2	5/4/98	-28.21	-21.49	-61.58	200.00	101.13	200.00	-21.35	-86.88	168.17	2.87
MWT5.1	6/3/98	114.92	118.18	4.08	-79.23	170.37	200.00	117.76	-18.25	189.79	-38.10
PXT0402	4/13/98	47.74	56.83	-45.89	-1.20	-30.69	200.00	56.04	-21.87	122.25	14.58
PXT0402	6/8/98	1.13	-11.18	19.05	-114.93	-200.00		-15.13	-100.23	200.00	20.56
RET3.1	5/6/98	175.51	48.40	186.78	196.29	200.00	200.00	81.21	6.54	200.00	84.85
RET4.3	6/10/98	67.78	93.66	-24.56	94.12	149.20		93.37	196.92	-122.83	34.08
RET5.2	4/22/98	37.73	11.38	41.73	15.49	200.00	200.00	13.09	-55.44	48.49	66.32
SBE5	5/14/98	35.43	38.71	200.00	200.00	200.00	200.00	37.50	9.28	115.93	70.01
TF3.3	6/10/98	-7.10	153.18	-97.28	-9.01	200.00		98.00	30.79	146.20	87.75
TF4.2	4/10/98	7.64	12.98	-61.70	200.00	200.00	-164.42	13.84	-36.61	71.00	12.84
TF5.5	5/20/98	-79.72	-28.22	-30.30	191.72	-200.00		-21.11	94.43	-55.12	58.35
WE4.2	4/6/98	-34.71	-5.71	-30.45	200.00	200.00	-30.51	-5.63	-25.73	200.00	44.82
XDE5339	4/13/98	111.65	113.95	-100.99	194.73	200.00	200.00	114.20	-3.39	176.52	12.91
XEA6596	6/1/98	179.18	181.64	174.05	179.15			180.42	118.70	199.90	-40.34
Average % D	ifference	46.51	65.44	-27.18	86.55	128.83	136.86	59.81	5.00	128.76	20.78

Table 4. Round 2 "First Ten" Split Samples (Elgin Perry 15:11 Thursday, January 28, 1999). Univariate Procedure, Variable = CVDIFF = Versar Coefficient of Variation minus ODU Coefficient of Variation.

#### Mom ents

N	65	Sum Wgts	65
Mean	-0.11482	Sum	-7.46348
Std Dev	0.261908	Variance	0.068596
Skewness	0.436674	Kurtosis	1.944496
USS	5.247104	CSS	4.390127
CV	-228.098	Std Mean	0.032486
T:Mean=0	-3.53456	Pr> T	0.0008
Num ^= 0	63	Num > 0	10
M(Sign)	-21.5	Pr>= M	0.0001 = Significant difference between the paired CV's w/ higher CV at ODU
Sgn Rank	-680	Pr>= S	0.0001 = Significant difference between the paired CV's w/ higher CV at ODU

# Quantiles (Def=5)

100% Max 75% Q3 50% Med 25% Q1 0% Min	0.643628 -0.02629 -0.07317 -0.23403 -0.72278	99% 95% 90% 10% 5%	0.643628 0.442719 0.00891 -0.413 -0.5563	1%	-0.72278
Range Q3-Q1 Mode	1.366407 0.207746 0				

#### Extremes

Five lowest and five highest observations (check for outliers)

5 Lowest (Obs #)	5 Highest (Obs #)
-0.72278 (152)	0.288705 (26)
-0.70642 (44)	0.442719 (121)
-0.66144 (196)	0.551221 (52)
-0.5563 (51)	0.601356 (142)
-0.5266 (6)	0.643628 (28)

Table 5. Results of Wilcoxon Signed Rank Analysis for Each Taxa in Round 2 "First Ten" Split Samples. The sign of the Signed Rank statistic indicates the direction of the difference. Positive values indicate that Versar estimates a greater abundance of the taxa while negative values indicate that ODU estimates a greater abundance of the taxa. For exploratory purposes, one might use a p-value of 0.05 bearing in mind that about 1 in every 20 tests will be a false positive by this criterion. (From Elgin Perry).

Wilcoxon Signed rank statistics by taxa with p-values.

TSN	NEWNODC	LIFES	TG TAX NAME	SGNRNK	р
064358	5001	97	POLYCHAETA	9.50	0.06250
069296	501401	BL	PISCICOLIDAE	0.50	1.00000
069459	51	97	GASTROPODA	5.00	0.12500
081388	551546	0	PISIDIIDAE	-3.00	0.25000
081388	551546	97	PISIDIIDAE	-1.50	0.50000
083833	6109	98	EUCLADOCERA	-0.50	1.00000
083833	6109	BL	EUCLADOCERA	10.00	0.10940
083873	61090201	BL	DAPHNIA	4.00	0.10940
083936	61090201	BL	30SMINA	-8.50	0.43160
083964	61090501		PODON	-2.50	0.43160
084195	6110	BL BL	OSTRACODA	8.50	0.82300
085761	61181701	12	CENTROPAGES	0.50	1.00000
085761	61181701	98	CENTROPAGES	0.50	1.00000
085780	61181801	12	DIAPTOMUS	3.00	0.25000
085780	61181801	98	DIAPTOMUS	3.00	0.25000
085848	61181902	12	PSEUDODIAPTOMUS	0.00	1.00000
085848	61181902	98	PSEUDODIAPTOMUS	-2.00	0.50000
085862	61182002	12	EURYTEMORA	13.00	0.03130
085862	61182002	98	EURYTEMORA	2.50	0.82030
085874	61182003	12	TEMORA	-4.00	0.25000
085874	61182003	98	TEMORA	-12.00	0.04690
086084	61182901	12	ACARTIA	10.50	0.03130
086084	61182901	98	ACARTIA	7.00	0.38280
086099	61183001	98	TORTANUS	0.50	1.00000
086110	6119	98	HARPACTICOIDA	-10.50	0.03130
086110	6119	BL	HARPACTICOIDA	5.50	0.31250
088599	61200501	BL	ERGASILUS	7.50	0.06250
088628	61200602	98	SAPHIRELLA	1.50	0.50000
088634	612008	12	CYCLOPIDAE	2.00	0.62500
088634	612008	98	CYCLOPIDAE	-7.50	0.06250
088634	612008	BL	CYCLOPIDAE	-2.00	0.62500
088802	61200901	98	OITHONA	-1.50	0.81250
089599	613402	11	3ALANIDAE	9.50	0.35940
089599	613402	17	3ALANIDAE	-14.00	0.01560
090054	61530115	BL	NEOMYSIS	3.00	0.25000
092120	6158	BL	ISOPODA	-5.50	0.31250
093294	6168	ВL	AMPHIPODA	9.50	0.06250
096383	61791103	31	PALAEMONETES	1.50	0.50000
097107	61792201	31	CRANGON	-0.50	1.00000
098763	61890206	31	HEXAPANOPEUS	-0.50	1.00000
098974	61890602	31	PINNOTHERES	-0.50	1.00000
	61HYDRAC	BL	HYDRACTINIA	1.50	0.50000
102467	6251	ВL	PLECOPTERA	0.50	1.00000
118831	6481	21	DIPTERA	3.00	0.25000
118831	6481	97	DIPTERA	0.00	1.00000
155457	770001	98	PHORONIDAE	-1.50	0.50000
159664	8412	98	APPENDICULARIA	-0.50	1.00000
167676	88357502	97	MORONE	-3.00	0.25000
171788	88470106	97	GOBIOSOMA	-0.50	1.00000
1,1,00	001,0100	<i>J</i> ,	00010001111	0.00	

Table 6. Comparison of number of taxa identified in each split sample in "First Ten." Life stages of individual taxon are not counted as separate taxa. However, if two or more closely related taxa are identified, they are kept separate (e.g. *Acartia* sp. and *Acartia tonsa* are counted as separate taxa). In the ODU count, "*Acartia* sp. j" and *Acartia* (ODU code 297) are assumed to be *Acartia tonsa* copepodite; "*Eurytemora* sp. j" and *Eurytemora* (ODU code 437) are assumed to be *Eurytemora affinis* copepodite; "*Mesocyclops* sp. j" is assumed to be *Mesocyclops edax* copepodite. Salinity: TF = tidal freshwater; OH = oligohaline; MH = mesohaline; PH = polyhaline.

Station (Salinity/Location)	Date	Rep	ODU Round 1	ODU Round 2	Versar Round 1
CB2.1 (OH/mainstem)	5/6/1998	1	17	12	23
CB3.3C (MH/mainstem)	5/5/1998	1	6	12	11
CB6.1 (MH-PH/mainstem)	5/18/1998	1	4	6	9
ET5.1 (TF/C hoptank R.)	4/7/1998	1	13	9	15
ET5.1 (TF/Choptank R.)	5/5/1998	1	19	15	19
LE3.6 (MH-PH/Rap pahanno ck R.)	6/1/1998	1	10	10	6
RET 3.1 (TF/Rappa hannock R.)	5/6/1998	1,2	10	13	21
RET 5.2 (PH/James R.)	4/21/1998	1	13	12	20
TF1.5 (TF/Patuxent R.)	6/8/1998	1	13	12	15
WE 4.2 (PH/York R.)	4/16/1998	1	10	14	12
Mean Number of T	axa		11.5	11.5	15.1

Table 7. Results of Wilcoxon Signed Rank Analysis for Each Taxa, Round 2 "Last Ten" Split Samples. The sign of the Signed Rank statistic indicates the direction of the difference. Positive values indicate that Versar estimates a greater abundance of the taxa while negative values indicate that ODU estimates a greater abundance of the taxa. Rankings with p-values of 1 or -1 indicate only one lab counted the identified species. For exploratory purposes, one might use a p-value ("Pr >= |S|") of 0.05 bearing in mind that about 1 in every 20 tests will be a false positive by this criterion. (From Elgin Perry, 9/1/99)

NEWNODC	LIFE_STG	NODCNAME	Sign Rank	Pr>= S
3702	98	HYDROIDA	0.5	1.0000
50	98	TROCHOPHORE	-3.0	0.2500
5001	97	POLYCHAETA	22.5	0.0039
5001	98	POLYCHAETA	-1.5	0.5000
51	98	GASTROPODA	-3.0	0.2500
55	97	BIVALVIA	5.0	0.1250
55	98	PELECYPODA	-1.5	0.5000
61090102	98	DIAPHANOSOMA BRACHYURUM	-3.0	0.3750
61090103	98	SIDA CRYSTALLINA	0.5	1.0000
61090201	98	DAPHNIA	0.5	1.0000
61090201	98	DAPHNIA LONGISPINA	2.0	0.6250
61090201	98	DAPHNIA PULEX	1.5	0.5000
61090301	98	BOSMINA LONGIROSTRIS	-7.0	0.3828
61090502	98	PODON POLYPHEMOIDES	-3.0	0.3750
61090601	98	LEPTODORA KINDTII	0.5	1.0000
61090701	98	ALONA	-0.5	1.0000
61090702	98	CHYDORUS	5.0	0.1250
61090705	98	LEYDIGIA QUADRANGULARIS	0.5	1.0000
61090805	98	ILYOCRYPTUS SPINIFER	5.0	0.1250
6110	98	OSTRACODA	-9.0	0.2500
61180505	12	PSEUDOCALANUS	-14.0	0.0156
61180505	98	PSEUDOCALANUS	-18.0	0.0078
61181701	12	CENTROPAGES	3.0	0.2500
61181701	98	CENTROPAGES HAMATUS	-0.5	1.0000
61181701	98	CENTROPAGES TYPICUS	0.5	1.0000
61181801	12	DIAPTOMUS	2.5	0.5625
61181801	98	DIAPTOMUS	-4.5	0.4375
61181902	12	PSEUDODIAPTOMUS CORONATUS	0.5	1.0000
61181902	98	PSEUDODIAPTOMUS CORONATUS	0.5	1.0000
61182002	12	EURYTEMORA	25.5	0.0059
61182002	98	EURYTEMORA AFFINIS	17.5	0.0840
61182003	12	TEMORA TURBINATA	0.5	1.0000
61182003	98	TEMORA LONGICORNIS	-0.5	1.0000
61182901	12	ACARTIA	4.5	0.3125
61182901	12	ACARTIA TONSA	1.5	0.5000
61182901	98	ACARTIA TONSA	6.0	0.3750
6119	98	HARPACTICOIDA	10.5	0.0313
61190502	98	CANUELLA ELONGATA	-7.5	0.0625
61191401	98	EUTERPINA ACUTIFRONS	-0.5	1.0000
61200801	12	HALICYCLOPS	-3.0	0.2500
61200801	98	HALICYCLOPS	-0.5	1.0000
61200802	12	CYCLOPS	10.5	0.0313
61200802	98	CYCLOPS BICUSPIDATUS	3.0	0.2500
61200802	98	CYCLOPS VERNALIS	0.5	1.0000
61200803	12	MESOCYCLOPS	-1.5	0.5000
61200803	12	MESOCYCLOPS EDAX	0.5	1.0000

61200803	98	MESOCYCLOPS EDAX	-1.5	0.5000
61200804	98	EUCYCLOPS AGILIS	0.5	1.0000
61200804	98	EUCYCLOPS SPERATUS	1.5	0.5000
61200807	98	TROPOCYCLOPS PRASINUS	0.5	1.0000
61200901	12	OITHONA	-0.5	1.0000
61200901	98	OITHONA	-1.5	0.5000
61200901	98	OITHONA COLCARVA	0.5	1.0000
613402	11	BALANIDAE	10.5	0.0313
613402	17	BALANIDAE	3.0	0.2500
61340201	11	BALANUS	-10.5	0.0313
61340201	17	BALANUS	-14.0	0.0156
615301	98	MYSIDAE	1.5	0.5000
61530115	93	NEOMYSIS AMERICANA	-3.0	0.2500
61530115	98	NEOMYSIS AMERICANA	7.5	0.0625
61530121	98	MYSIDOPSIS BIGELOWI	0.5	1.0000
6154	98	CUMACEA	0.5	1.0000
61540508	98	OXYUROSTYLIS SMITHI	0.5	1.0000
61691502	98	COROPHIUM LACUSTRE	3.0	0.2500
61692107	98	GAMMARUS	3.5	0.5625
61693708	98	MONOCULODES	7.5	0.0625
61792201	31	CRANGON SEPTEMSPINOSA	-3.0	0.2500
61792201	98	CRANGON SEPTEMSPLNOSA	1.5	0.5000
64890502	98	CHAOBORUS PUNCTIPENNIS	0.0	1.0000
648933	21	CHIRONOMIDAE	1.5	0.5000
648933	97	CHIRONOMIDAE	7.5	0.0625

Table 8. Comparisons of Taxa Totals for Round 2, Last Ten Samples, show count increases due to the addition of a 64 micron mesh sieve to the CVS method used by ODU. However, ODU counts are still lower than Versar counts. Features: S = small, M = medium, L = large, VL = very large. Versar Total: calculated total number for this taxa in all ten split samples. ODUwout - Total: calculated total for this taxa in all ten split samples, method is CVS without 64 micron mesh seive ("old" method used prior to 1999). ODUw - Total: calculated total for this taxa in all ten split samples, method is CVS with 64 micron mesh seive. % Difference: (VersarTotal) minus (ODUw - Total) divided by the mean of [(VersarTotal) and (ODUw-Total)] and then multiplied by 100, where a positive % means Versar counts more individuals and a negative percent means ODU counts more individuals. Z: general results of z-score statistical analysis on paired split samples (from Appendix D or Table 9), where NS = no/few significant differences observed and/or no preponderance of higher counts by one laboratory; S(V) = many significant differences observed with a preponderance of higher Versar counts; rare = split counts were mostly  $\leq 2000/sample$ , so % error is relatively high; "-" = z-score not calculated. Conversion factor: multiplier that could be used to convert pre-1999 ODU counts to values comparable to Versar counts. A factor is only calculated for individual taxa with grand totals (total in 10 split samples) of >30,000 in both of the splits.

Features	Taxa	VersarTotal	ODUwout - Total	ODUw - Total	% Difference	Z	Conversion Factor
	Total Cladocera	188,308	84,322	125,666	39.9	-	
S-M round	Bosmina	77,850	65,966	87,278	-11.42	NS	1.1802
S-M round	Chydorus/chydorids	82,800	1,024	17,408	130.51	rare	
S-L round	Daphnia spp.	14,600	3,090	5,906	84.79	rare	
M round	Podonidae (Podon & Evadne)	12,800	13,952	14,272	-10.87	rare	
	all other Cladocera	258	290	802		rare	
1	I =	000 540	1 004 770	000 000	1 22 52		
	Total Miscellaneous	328,513	204,773	222,309	38.56	-	
soft	Mollusc & annelid larvae (polychaetes, gastropod, pelecypod)	7,621	20,362	21,130	-93.97	rare	
S-M round	Ostracods	27,450	50,679	64,247	-80.26	rare	
S-M round	Barnacle cypris	6,200	10,184	10,504	-51.53	rare	
S	Barnacle nauplii	287,242	123,548	126,428	77.75	S(V)	2.3249
	Total Copepods	7,842,884	2,872,598	3,797,622	69.5	-	
VL	Eurytemora adults	1,756,400	1,549,058	1,557,250	12.02	S(V)	1.1339
M	Eurytemora copepodites	5,794,800	963,947	1,533,803	116.28	S(V)	6.0115
<u>L</u>	Acartia adults	98,400	98,650	100,698	-2.31	S(V)	0.9731
S	Acartia copepodites	69,435	17,552	30,896	76.82	S(V)	3.9560
	Other Calanoids ad&cop	22,272	189,621	437,781	-180.64	-	
	All Calanoids adults and copepodites	7,738,907	2,818,828	3,660,428	71.56	-	
	Mesocyclops	402	4,228	<b>7</b> ,044	-178.4	rare	
S	Oithona	800	1,281	36,129	-191.33	rare	
	All Cyclopoids adults and copepodites	67,799	22,944	105,856	-43.83	-	0.6405
	All Harpactacoids adults and copepodites	36,178	30,826	31,338	14.34	-	1.1736
	GRAND TOTALS OF TEN SAMPLES	8,359,705	3,161,693	4,145,597	67.40	-	

NODCGRP-barnacle nauplii

Table 9. Z-Scores for Barnacle Nauplii, Selected Copepods, Small-Round Taxa, and Polychaete Larvae. The estimated total and the variance estimate for four size-based or shape-based groupings\* were computed. The methods for these computations are described in Appendix B. The analysis were run on the NEWNODC code field which contains higher level (more general) taxonomic codes. The estimated total and it's sampling variance were computed for each sample and grouping (and sieve in the case of ODU). For ODU, estimated counts and their variances were summed across sieves. A z-score was calculated from the variances and used to compare the labs on a sample by sample/taxa by taxa basis. Variables names are: STATION, DATE, VETOT = Versar Estimated Total, OETOT = ODU Estimated Total, VEVTOT = Versar Estimated Variance of Total, OEVTOT = ODU Estimated Variation, OCV = ODU Coefficient of Variation, DIFF and VDIFF are calculation steps, and Z = Z-score for difference of counts. A z-score of 2 (**bold** numbers below) has about a 1/20 chance of occurring by accident. (From Elgin Perry 10/28/99)

NODCGRP-	-Daina	cre naup	,							
STATION	MONTH	VETOT	OETOT	TOTVEV	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	1600	0	638400	0	0.49937	•	1600	640000	2.00000
CB5.2	3	82000	32321	81918000	26717632	0.11038	0.15992	49679	108749953	4.76385
ET5.2	4	4400	1032	1755600	261176	0.30113	0.49521	3368	2022208	2.36842
LE2.2	3	192000	77842	383808000	132042768	0.10204	0.14762	114158	516120610	5.02494
RET2.2	4	0	8192	0	67100672	•	0.99994	-8192	67108864	-1.00000
TF1.5	4	400	0	159600	0	0.99875	•	400	160000	1.00000
TF1.7	3	0	1	0	0	•	0.00000	-1	1	-1.00000
TF1.7	4	7200	7040	2872800	2860160	0.23541	0.24023	160	5747200	0.06674
NODCGRP=	_		ND Troc	hophores VEVTOT	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	2000.00	6200	798000.00	12576824	0.44665	0.57200	-420C.00	13383024.00	-1.14808
CB5.2	3	1000.00	0	199000.00	0	0.44609	•	1000.00	200000.00	2.23607
ET5.2	4	235.29	768	55128.03	195840	0.99787	0.57622	-532.71	251971.32	-1.06124
LE2.2	3	2000.00	2560	798000.00	4322816	0.44665	0.81216	-560.00	5125376.00	-0.24736
TF1.5	3	1600.00	0	1278400.00	0	0.70666	•	1600.00	1280000.00	1.41421
TF1.5	4	400.00	8	159600.00	24	0.99875	0.61237	392.00	160032.00	0.97990
TF1.7	3	400.00	0	159600.00	0	0.99875	•	400.00	160000.00	1.00000
TF1.7	4	400.00	0	159600.00	0	0.99875	•	400.00	160000.00	1.00000

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TF2.3 4 250.00 0 62250.00 0 0.99800 . 250.00 62500.00 1.00000

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### NODCGRP="Round" organisms

STATION	MONTH	VETOT	OETOT	VEVTOT	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	1600	5130	638400	6286346	0.49937	0.48874	-3530	6931476	-1.3408
CB5.2	3	800	2112	159200	2097088	0.49875	0.68567	-1312	2259200	-0.8729
ET5.2	4	2000	3634	798000	4584272	0.44665	0.58918	-1634	5387906	-0.704C
LE2.2	3	11600	12032	4628400	6738176	0.18546	0.21574	-432	11390208	-0.1280
RET2.2	4	250	16576	62250	268431168	0.99800	0.98841	-16326	268510244	-0.9963
TF1.5	3	99200	7 4 4 4	79260800	2833396	0.08975	0.22612	91756	82200840	10.1204
TF1.5	4	49450	103196	19693050	291597640	0.08974	0.16547	-53746	311443336	-3.0455
TF1.7	3	7600	4224	3032400	1412992	0.22913	0.28141	3376	4457216	1.5991
TF1.7	4	750	773	186750	294144	0.57619	0.70162	-23	482417	-0.0331
TF2.3	4	41708	75444	16563300	373692850	0.09758	0.25623	-33736	390373302	-1.7075

### NODCGRP=Selected Copepods

STATION	MONT	H VETOT	OETOT	VEVTOT	CEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	186705.88	301702	365579384	1064396614	0.1024	0.1081	-114996.12	1430464405	-3.0405
CB5.2	3	50612.00	23200	10069400	7881056	0.0627	0.1210	27412.00	18024268	6.4567
ET5.2	4	220400.00	143701	423939600	282326648	0.0934	0.1169	76699.00	706630349	2.8853
LE2.2	3	81200.00	39459	32398800	52258334	0.0701	0.1832	41741.00	84777793	4.5334
RET2.2	4	452800.00	1054772	1707067200	31294744624	0.0912	0.1677	-601972.00	33003319396	-3.3136
TF1.5	3	359400.00	74340	542860600	109403948	0.0648	0.1407	285060.00	652698288	11.1578
TF1.5	4	225850.00	370573	352476650	1128880756	0.0831	0.0906	-144723.00	1481953829	-3.7594
TF1.7	3	112235.29	166927	88903128	292385792	0.0840	0.1024	-54691.71	381568082	-2.7999
TF1.7	4	284051.00	268863	977148450	424666624	0.1100	0.0766	15188.00	1402367988	0.4056
TF2.3	4	81802.00	583932	63848200	7262394360	0.0976	0.1459	-502130.00	7326908294	-5.8662

# \* Grouping Assignments were made to the taxa identified by Versar and by ODU as follows:

Versar				Old Dominion	Old Dominion University						
NEWNODC	NODC Code L:	ife_Stg	Name	NEWNODC	NEWNODC NODC Code Life_Sto		Name				
Balanidae	613402	11	BALANIDAE	Balanidae	61340201	11	BALANUS				
PolyTroc	5001	97	POLYCHAETA	PolyTroc	50	98	TROCHOPHORE				
Round	61090102	98	DIAPHANOSOMA	PolyTroc	5001	97	POLYCHAETA				
Round	61090103	98	SIDA CRYSTALLINA	PolyTroc	5001	98	POLYCHAETA				
Round	61090201	98	DAPHNIA	Round	61090102	98	DIAFHANOSOMA BRACHYURUM				
Round	61090301	98	BOSMINA LONGIROSTRIS	Round	61090201	98	DAPHNIA LONGISPINA				
Round	61090502	98	PODON POLYPHEMOIDES	Round	61090301	98	BOSMINA LONGIROSTRIS				
Round	61090601	98	LEPTODORA KINDIII	Round	61090502	98	PODON POLYPHEMOIDES				
Round	61090702	98	CHYDORUS	Round	61090701	98	ALONA				
Round	61090705	98	LEYDIGIA QUADRANGULARIS	Round	6110	98	OSTRACODA				
Round	61090805	98	ILYOCRYPTUS SPINIFER	S Cope	61180505	12	PSEUDOCALANUS				
Round	6110	98	OSTRACODA	S_Copc	61180505	98	PSEUDOCALANUS				
S_Cope S Cope	61180505 61180505	12 98	PSEUDOCALANUS MINUTUS PSEUDOCALANUS MINUTUS	S Cope	61181701	12	CENTROPAGES				
S_Cope S_Cope	61181701	12	CENTROPAGES	S Cope	61181701	98	CENTROPAGES HAMATUS				
S_Cope	61181701	98	CENTROPAGES HAMATUS	S Cope	61181801	12	DIAPTOMUS				
S_Cope	61181801	12	DIAPTOMUS	S Cope	61181801	98	DIAFTOMUS				
S_cope	61181801	98	DIAPTOMUS	S Cope	61182002	98	EURYTEMORA AFFINIS				
S_Cope	61181902	12	PSEUDODIAPTOMUS CORONATUS		61182003	98	TEMORA LONGICORNIS				
S Cope	61181902	98	PSEUDODIAPTOMUS CORONATUS	S Cope	61182901	12	ACARTIA				
S Cope	61182002	98	EURYTEMORA AFFINIS	S Cope	61182901	98	ACARTIA TONSA				
S Cope	61182003	12	TEMORA TURBINATA	S Cope	61190502	98	CANUELLA ELONGATA				
S Cope	61182901	12	ACARTIA TONSA	S Cope	61191401	98	EUTERPINA ACUTIFRONS				
S_Cope	61182901	98	ACARTIA TONSA	S Cope	61200801	12	HALICYCLOPS				
S_Cope	6119	98	HARPACTICOIDA	S Cope	61200801	98	HALICYCLOPS				
S_Cope	61190502	98	CANUELLA ELONGATA	S_Cope	61200803	12	MESOCYCLOPS				
S_Cope	61200801	98	HALICYCLOPS	S_Cope	61200803	98	MESCCYCLOPS EDAX				
S_Cope	61200802	12	CYCLOPS	S_Cope	61200901	12	OITHONA				
S_Cope	61200802	98	CYCLOPS BICUSPIDATUS	S_Cope	61200901	98	OITHONA				
S_Cope	61200803	12	MESOCYCLOPS EDAX	2_cobe	01200901	30	OTTHONA				
S_Cope	61200803	98	MESOCYCLOPS EDAX								
S_Cope		98	EUCYCLOPS AGILIS								
S_Cope	61200807	98	TROPOCYCLOPS PRASINUS								
S_Cope	61200901	98	OITHONA COLCARVA								

Table 10. Comparisons of total mesozooplankton counts made with the "old" and "new" CVS methods in Round 2. Numbers are total, non-normalized numbers per sample jar.

				"Old" CVS Method	"New" CVS Method	
Batch	Station	Rep	Month	w/out 64u sieve	w/ 64u sieve	New/Old
First Ten	CB2.1	1	5	65,315	73,379	1.1235
First Ten	CB3.3C	1	5	361,277	426,301	1.1800
First Ten	CB6.1	1	5	151,632	166,096	1.0954
First Ten	ET5.1	1	4	92,486	150,854	1.6311
First Ten	ET5.1	1	5	208,535	511,639	2.4535
First Ten	LE3.6	1	6	9,860	15,420	1.5639
First Ten	RET3.1	1	5	2,726,006	2,982,006	1.0939
First Ten	RET3.1	2	5	3,168,902	3,379,590	1.0665
First Ten	RET5.2	1	4	267,207	424,647	1.5892
First Ten	TF1.5	1	6	311,685	414,213	1.3289
First Ten	WE4.2	1	4	63,030	82,230	1.3046
SecondTen	CB2.2	1	3	365,527	398,852	1.0912
SecondTen	CB5.2	1	3	58,468	60,356	1.0323
SecondTen	ET5.2	1	4	169,439	213,471	1.2599
SecondTen	LE2.2	1	3	160,856	174,296	1.0836
SecondTen	RET2.2	1	4	1,605,108	1,620,084	1.0093
SecondTen	TF1.5	1	3	89,060	98,660	1.1078
SecondTen	TF1.5	1	4	288,976	344,784	1.1931
SecondTen	TF1.7	1	3	120,767	137,535	1.1388
SecondTen	TF1.7	1	4	160,454	241,458	1.5048
SecondTen	TF2.3	1	4	151,950	864,654	5.6904
					AVERAGE	1.5019

Table 11. Approximate range of body lengths <sup>a</sup> and widths <sup>b</sup> for some taxa found in Chesapeake Bay. A typical length to width (L:W) ratio for each taxa was determined from drawings and photographs in the available literature. <sup>a</sup> Length and width estimates do not include the dimensions of antennae, spines, caudal rami, etc.

	Length ( )	Width ( )	L:W ratio
Adult Copepods			
<i>Eurytemora affinis</i> adult	1,400-1,800	350-450	4:1
Acartia tonsa female adult	1,250-1,500	270-325	4.6:1
Acartia tonsa male adult	1,000-1,150	215-250	4.6:1
Pseudocalanus adult	700-1,500	175-375	4:1
Copepodites			
Acartia copepodite stages I-III	350-570	95-150	3.75:1
Eurytemora affinis copepodite I-V	475-1,275	135-365	3.5:1
Cladocera (immatures & adults)			
Podon polyphemoides	200-800	120-470	1.7:1
Evadne nordmanni	200-1000	120-590	1.7:1
Bosmina longirostris	180-2000	140-1,540	1.3:1
Daphnia pulex	50-2,200	30-1,220	1.8:1

a derived from several sources including 1) Todd and Laverack. 1991. Coastal marine zooplankton: a practical manual for students. Cambridge University Press. 2) Conover, R.J. 1956. Comparative development of *A. clausi* and *A. tonsa*. Bull. Bingham Oceanogr. Coll. 15:156-233. 3) Wilson, C. B. 1932. The copepods of the Woods Hole Region, Massachusetts. 4) Edmondson, W. T. (ed.) 1959. Freshwater Biology, 2<sup>nd</sup> edition. John Wiley & Sons, Inc. 5) Pennak, R. W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, Inc. 6) Dodson, S. I. 1981. Morphological variation of *Daphnia pulex* Leydig (Crustacea:Cladocera) and related species from North America. Hydrobiologia 83:101-114. 7) Huff (Appendix 15)

<sup>&</sup>lt;sup>b</sup> derived from length and L:W ratio

Table 12. Mean and range of raw counts tallied for taxa identified in the Round 2 - Last Ten split samples. Versar raw counts tended to be higher than ODU raw counts for the relatively common taxa. This observation reflects Versar's laboratory objective of counting at least 60 individuals of the dominant and subdominant taxa which gives these counts an error level of about  $\pm 25\%$  or better (p<0.05). It reflects ODU's laboratory objective of counting 20-42 individuals of the dominant and subdominant taxa to obtain an error level of  $\pm 35\%$  (p<0.05). See Appendix A for more detail. Versar and ODU raw counts were roughly equivalent for the moderately abundant taxa. Size categories of adults are given for comparisons purposes. The categories are based on the mean adult lengths obtained from literature values, and are determined by the following size (length) fractions: small (S) is < 500 , medium (M) is 500 - 800 , large (L) is 800 - 1,200 , and very large (VL) is > 1,200 .

	Adult Size	ODU		Versar		
	Category	Mean Cnt	Range	Mean Cnt	Range	
"Common" taxa						
Acartia ton sa adults	L	26	2-48	47	6-74	
Acartia copepodites		32	19-52	31	1-82	
balan us na uplii		28	1-61	35	1-96	
Eurytemora affinis adults	VL	73	4-121	95	2-200	
Eurytemora copepodites		72	1-140	185	4-522	
"Rarer" taxa						
Bos min a long irostris	S	18	1-62	28	1-91	
Podon polyphemoides	М	15	1-40	11	1-29	
Polychaeta		11	2-28	2	1-5	
Daphnia	L-VL	11	1-32	3	1-12	
Ostracoda	mixed	9	2-17	10	1-23	
Neomysis americana	VL	8	1-18	46	11- <b>7</b> 5 Whole C	nt

# Appendix A: Pre-1998 Mesozooplankton Methodologies

	MARYLAND	VIRGINIA
NUMBER OF STATIONS	16 (3 MAINSTEM, 9 TRIB, 4 SEASONAL)	14 (4 MAINSTEM, 10 TRIB)
SAMPLE COLLECTION TYPE	COMPOSITE	COMPOSITE
FIELD COLLECTION PROCEDURES	Two stepped oblique, replicate tows are taken at each station through the entire water column. Steps are taken in 1-4 meter increments depending on total station depth. There are always 5 step levels per station. Tows last between 5 to 10 minutes depending on zooplankton abundance. One of the paired nets is used for taxonomic purposes (counting), the other for biomass measurements. The count sample is preserved.	Monthly net collections of 5 minutes oblique tows, over the water column, are made at 15 stations (located in the Bay and Tributaries) using a paired ½ meter bongo frame containing a set of 202 micron mesh nets, each equipped with flow meter. After the tow, each net will be washed down to collect the sample into bottles which are preserved with 7% buffered formalin. These two paired samples represent replicate samples for that station. Flowmeter readings were taken before and after each tow to determine the volume of water filtered
MESOZOOPLANKTO N ENUMERATION TECHNIQUE	A hierarchical counting technique is employed to obtain density estimates. This procedure consists of first counting at least 60 individuals of the most dominant forms (e.g. Acartia tonsa) in a small subsample (usually 1 - 2 milliliters), followed by 5- and 10- milliliter subsamples from which all species that had counts less than 60 in the previous subsample are counted.	Processing and analysis of samples is conducted by the coefficient of variation stabilizing method (Alden et al. 1982). Size fractionation of each sample produces 5 size classes (200, 300,600, 850, 2000 microns). Size classes in which the organisms are too numerous to count in their entirety are split with a folsom plankton splitter until an appropriate sample size is reached for statistically valid counts of the dominant species. The chosen error level of 35% requires that each species of interest be counted to achieve a range of between 20 and 42 individuals in any given split. Species observed to be subdominant in the final split are counted until they have achieved the range for the 35% error level. Taxon abundance is recorded as numbers per unit volume.

	MARYLAND	VIRGINIA
MESOZOOPLANKTO N BIOMASS DETERMINATION TECHNIQUE	Dry weights and ash weights are measured by gravimetric methods for detritus-free samples. Samples containing detritus are not processed and are disposed of after the final report is completed. A regression-based computer program is used to estimate mesozooplankton biomass in samples containing detritus. In detritus contaminated samples values for dry weight are based on the known weight (from literature or by weighing of organisms) multiplied by the number present and summed across all taxa in the sample.	ONLY DRYWEIGHT DETERMINATION IS PERFORMED. NO CURRENT METHODOLOGY ON FILE. Data are NOT CONSISTENTLY COLLECTED.
BIOVOLUME DETERMINATION TECHNIQUE	Cnidarians (true jellyfish, hydromedusae) and ctenophores (comb jellies) are separated from the samples in the field after sample preservation. The separate settled volumes of the jellyfish are then measured and recorded. Settled volumes are measured from the correlative count sample for each biomass sample. Samples are poured into Imhoff cones and left undisturbed for 2 - 4 days as plankton settles to the bottom of the cone. After settling time, the reading(top of settled material) is recorded in the lab notebook.	NO METHODOLOGY ON FILE . STARTED REPORTING SETTLED VOLUMES AS OF JAN 96.
ARCHIVE SAMPLES	?	?
NUMBER OF OBSERVED TAXA	157	451
PRESERVATIVES	FORMALIN	Lugol's Solution

	MARYLAND	VIRGINIA
METHOD OF CALCULATION FOR TAXON DENSITY	DENSITY = A * (B/(C * FVOL_M3)), where DENSITY = density in numbers per cubic meter A = number of individuals counted in the subsample, B = volume in milliliters of sample from which subsamples are taken, C = subsample volume in milliliters, FVOL_M3 = volume of water filtered by the bongo nets in cubic meters = (3.14*(r**2)) * (Y*(26,873/999,999)), where r = radius of the bongo net, Y = flowmeter count (i.e. difference between beginning count and end count, 26,873 = a rotor constant equal to the standard distance traveled in meters for 999,999 revolutions of the flow meter, 999,999 = the maximum revolutions that can be read by flowmeter	The following formula is used to calculate distance traveled by the bongo net during a tow.  DISTANCE=(STOP-START)*BLADE CONSTANT Where STOP is the number of revolutions recorded on the bongo net flow meter at the end of the tow, START is the number of revolutions on the meter at the beginning of the tow, DISTANCE is the distance traveled by the bongo net during the tow. The blade constant is equal to 26873/999999. The sample volume is calculated using the following equation: SAMPLE VOLUME= DISTANCE*AREA where AREA + 0.18776 square meters. Densities are first calculated for each size class and then a total density is calculated. The size classes range from 200 to 2000 microns and represent the sieve sizes used to separate organisms into categories prior to identification and enumeration. SC2000= ((2**SC2000S)*SC2000C)/VOLSC850 = ((2**SC300S)*SC850C)/VOLSC600 = ((2**SC300S)*SC300C)/VOLSC200 = ((2**SC300S)*SC300C)/VOLSC200 = ((2**SC2000S)*SC200C)/VOLT_DENS= SC2000+SC850+SC600+SC300+SC200SC= Density of taxa)

# Appendix B: Methods for Comparing Results from Two Laboratories Participating in Plankton Split Sample Studies

### Elgin Perry

The objective of the comparison is to determine is the differences in the estimated sample counts computed by two laboratories consistently differ by more than the chance variation that results from the original split that divides the sample for the two laboratories coupled with the variance that results from subsampling within the laboratories. This evaluation will proceed on two levels. The first level will compare the counts obtained by the laboratories on a sample by sample basis for each taxonomic group. The second level will consider the cumulative evidence across all samples.

For each sample, it is possible to compute the sampling variance of the estimated count. This computation requires that each organism in the sample have equal probability of being selected by the lab's subsampling procedure and that probability must be equal to the proportion of the sample that is fully enumerated by the lab. In addition, the original split that divides the sample for the two labs introduces some variation between the counts that are obtained by the two labs and this variance will also contribute to differences between the labs. The details of computing this variance estimate are given below in "Formulation of Sampling Variance Estimate and Splitting Variance Estimate." By combining the subsampling variance and the splitting variance, it is possible to estimate the variance of the difference between the two labs. The difference between the two labs divided by its standard deviation forms a z-score the absolute value of which should exceed 1.96 only 5% of the time if the null hypothesis of no relative bias between the labs is true. This z-score will be the basis of comparing the labs on a sample by sample basis.

In the second level of analysis, the combined evidence of all the samples will be assessed using the Wilcoxon signed rank test. This test requires a minimum of distributional assumptions. The data should be from a continuous distribution to minimize ties and the data should be from a symmetric distribution. Some data transformation may be required to achieve symmetry. From this test we will learn if there is a consistent bias of one lab relative to the other based on all the samples.

General guidance for interpreting the results from these analyses is as follows. If the z-score test shows occasional differences and these differences are not always in the same direction and the Wilcoxon test does not confirm a consistent bias, we will conclude that the methods and taxonomy employed by the labs are comparable. If the majority of z-score tests indicate differences and these differences are not in the same direction and the Wilcoxon test does not confirm a consistent bias, it is likely that some source of extra-binomial variance is affecting the sample processing and this source of variance should be identified and removed. If the z-score tests indicate a high frequency of differences between labs and these differences are consistently in one direction and the Wilcoxon test confirms that there is a consistent bias, we will conclude that data from the labs are not comparable. There are other outcomes that may warrant attention. If for example a low frequency of differences are identified by the z-score test which are all it the same direction and all from a single salinity zone, this may indicate a taxonomy problem in that salinity zone.

To lend perspective to the differences between labs, this difference may be expressed as a percentage of the average of the two labs.

percent difference = 
$$200 \left[ \frac{n_1 - n_2}{n_1 + n_2} \right]$$

Where:  $n_1 =$ the count for lab 1, and  $n_2 =$ the count for lab 2

If these percentages are combined over taxonomic groups or across samples, a weighted averaging formulation will be used to insure that large percentages that result from small numbers of organisms do not distort the results.

#### Formulation of Sampling Variance Estimate and Splitting Variance Estimate

Let p be the proportion fo the sample that is enumerated. N is the number of organisms in the sample.

n is the number in the subsample that are enumerated.

Rules for evaluating moments applied to the binomial distribution tell us that

$$Var(n) = Npq$$

Where q = (1-p).

N is unknown, but can be estimated by

$$\hat{N}=n/p$$

Substituting this into the equation above yields

$$s_n^2 = \hat{N}pq = (n/p)pq = nq$$

When comparing counts between laboratories, it is the sampling variance of  $\hat{N}$  that is needed. When a random variable, for example n is multiplied by a constant, for example 1/p then the variance of this product is obtained by multiplying the variance of the random variable  $(s_n 2)$  by the square of the constant. Applying this rule we obtain

$$var(\hat{N}) = s_n^2 \left(\frac{1}{p}\right)^2 = \frac{nq}{p^2} = s_N^2$$

This quantifies the variance due to subsampling within labs. It remains to quantify the variance due to the original split that divided the sample between the labs. At this point we add a subscript I to distinguish between labs. That is:  $\hat{N}_i$  is the estimated count from lab i and  $s_{N_i}^2$  is the estimated variance for lab i.

Assume that there are N total organisms in a sample to be split. As a result of the split, x organisms go to one lab and N-x go to the other. The difference between the two labs is 2x-N. If the p of the original split was 0.5 then the E(2x-N) = 0 and the variance of 2x-N is

$$Var(2x-N) = 4 Var(x) - Var(N) = 4N(\frac{1}{2})(\frac{1}{2}) = N.$$

Our best estimate of the total number of organisms before the split is  $\hat{N} = \hat{N}_1 + \hat{N}_2$ .

Combining the results from above, the estimate of the variance of the difference between labs is given by  $s \frac{2}{diff} = \left(\hat{N}_1 + \hat{N}_2\right) + s \frac{2}{N_1} + s \frac{2}{N_2} = \frac{n_1}{p_1} + \frac{n_2}{p_2} + \frac{n_1 q_1}{p_1^2} + \frac{n_2 q_2}{p_2^2}$ 

# Appendix C: Letter from Versar to Maryland Department of Natural Resources following March 10-12, 1999 meeting at Old Dominion University.

April 6, 1999

Bruce Michael
Tidewater Ecosystem Assessments
Maryland Department of Natural Resources
Tawes State Office Building, D-2
580 Taylor Avenue
Annapolis, Maryland 21401

Dear Bruce:

On March 10, 1999 Craig M. Bruce from Versar, Inc visited the zooplankton laboratory at Old Dominion University (ODU) for three days discussing techniques, taxonomy, nomenclature, and ways to make laboratory processing more compatible between the Maryland and Virginia programs. The purpose of this letter is to summarize the results of this meeting and to identify solutions.

Historically, Versar and ODU identified rotifers such as Brachionus sp. to the genus level. While they are seen in samples collected with a 202- m mesh net, the microzooplankton program generates more accurate numbers for this taxa. Therefore, both organizations have agreed stop counting rotifers in the mesozooplankton samples. Several in-house taxanomic differences were identified at ODU for cyclopoids, isopods, and amhipods. The differences were related to the level of identification and it was resolved that when possible animals should be identified to genera (e.g., gammarus vs. unidentified amphipod).

We determined that Versar had been misidentifying barnacle cypris (eggs) as ostracods in high salinity stations. Although the taxonomic issue has been resolved beginning with March 1999 samples, Versar will query historic data to determine when and where barnacle nauplii and the misidentified ostracods were present together. In this situation it is most likely that organisms identified as ostracods were barnacle cypris. If this is the case we will recode the species as barnacle cypris. Versar will contact Jackie Johnson to correct the data.

One of the ODU taxonomist had been misidentifying Eurytemora sp. as Temora sp. at some stations. This error was most likely due to inexperience. The taxonomist presently can identify the difference between the genera. Versar has not reported Eurytemora americana whereas ODU has. The differences between E. affinis vs. E. americana were discussed based on descriptions in C. Wilson's Copepods of Woods Hole Region Massachusetts. In lieu of the descriptions, it appears that ODU has been misidentifying E. affinis as E. americana; however, both groups will be alert for E. americana. The taxonomic issue has been resolved (E. affinis had four segments on the urosome while E. americana has five segments).

The lumping of E. affinis and E. hirundoides into E. affinis was questioned. However, according to Frank Ferrari at the Smithsonian Institution, the two species names are now considered to be synonymous. George Mateja is going to follow up on this reclassification by asking Paul Fofonoff of the Smithsonian Environmental Research Center to evaluate several specimens.

We discussed the nomenclature changes such as Cyclops vernalis to Acanthocyclops vernalis and Cyclops bicuspidatus to Diacyclops thomasi. Versar has changed the names in its database to the new designations.

The consistency of identification levels between Versar and ODU was discussed. It was agreed that:

- The most common species of Daphnia will be identified to species level.
- The most common species of Harpacticoid will be identified to genus and/or species level.
- The most common Diptera will be identified to family or genus.
- The most common Amphipod will be identified to family or genus.
- Crab zoea and megalops will be identified to species level.
- Specific larval stages (e.g. trochophore and spionidae) will not be deffientiated. Instead they will be reported as polychaete larvae.

Versar does not currently count fish eggs, fish larvae, or protochordates; ODU does count these organisms but the information is not reported. Versar will continue not to count these organisms based on an earlier decision by the Chesapeake Bay Program that the current field gear does not effectively sample these organisms.

We noticed that ODU did not count Bos min a long irostris in sample W E4.2 during Round 1 of the split sample but reported a density of 326 m³ in Round two of the comparison. ODU felt that the occurrence of B. longirostris was possibly due to sample contamination during sample sieving/splitting. We decided that for Round 3 of the comparison, 10 new samples would be examined. The 10 new samples will be used to rule out any previous sample contamination and should better represent the consistency of taxonomic techniques. All 10 samples will be from the regular Maryland collections to avoid additional costs to the program split. The first half of the new samples will be taken from March and the second half of the samples will be taken from April.

The list of specimens in Table 1 were either examined or discussed during our meeting.

Table 1. List of species discussed at ODU and Versar meeting in March 1999								
Acartia tonsa	Centropages hamatus							
Ameroculodes species complex	Centropages typicus							
Argulus	Cha obo rus p unc tipen nis							
Barn acle cypris	Chironomidae larvae							
Barn acle naup lii	Crab megalops unid.							
Bivalvia	Crab zoea unid.							
Brachionus	Crangon septemspinosa							
Cumacea	Neomysis americana							
Cyclops bicuspidatus (Diacyclops thomasi)	Oithona							
Cyclops vernalis (Acanthocyclops vernalis)	Ostracoda							
Dap hnia	Paguridae							
Diaphansoma sp.	Pagurus longicarpus							
Diaptomus	Pag urus pollica ris							
Ergasilus	Palaemonetes sp. zoea							
Eurytem ora affinis	Phronidae							
Hexarthra sp.	Podon polyphemoides							
Hydroid	Polychaete larvae							
llyocryptus spinifer	Pseudodiaptomus coronatus							
Isopoda	Rhithropanopeus							
Labidocera aestiva	Saphirella							
Leptodora kindtii	Gammarus							
Mesocyclops edax	Tem ora turbina ta							
Moina	Tortanus discaudatus							
Mysid opsis	Gastropod unid.							
Mysidopsis almyra	Halicyclops							
Mysidopsis bigelowi	Tropocyclops prasinus							
	Harpac tic oid							

The meeting between Versar and ODU was very productive. However, given laboratory personnel turn over rates (especially ODU graduate students) annual workshops to discuss taxonomy and laboratory

counting techniques should be continued to ensure the continued development of a bay-wide zooplankton indicators.

Sincerely,

### William Burton

cc: C. Bruce

C. Buchanan F. Jacobs File: 4337-101

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# Appendix D: Z-Score Statistical Analysis of Round 2 Last Ten Split Samples

The estimated total and the variance estimate for each taxonomic group and sample were computed. The methods for these computations are described in Appendix B. The estimated total and it's sampling variance were computed for each sample, taxa, and lifestage (and sieve in the case of ODU). For ODU, estimated counts and their variances were summed across sieves. A z-score was calculated from the variances and used to compare the labs on a sample by sample/taxa by taxa basis. Variables names are: STATION, DATE, VETOT = Versar Estimated Total, OETOT − ODU Estimated Total, VEVTOT − Versar Estimated Variance of Total, OEVTOT − ODU Estimated Variance of Total, VCV − Vesar Coefficient of Variation, OCV = ODU Coefficient of Variation, Z = Z-score for difference of counts. A z-score of 2 has about a 1/20 chance of occurring by accident and a z-score of 3 has about a 1/100 chance of occurring by accident. Z-score ≥ 2.0 are **bolded**. (From Elgin Perry 9/1/1999)

NEWNODC		E_TAXON	STATION	MONTH	VETOT	ОЕТОТ	VEVTOT	OEVTOT	VCV	OCV	Z
3702	STG 98	HYDROIDA	TF2.3	4	2.0	0.0		0.0			
50	98	TROCHOPHORE	LE2.2	3	0.0	2048.0	0.0	4192256.0		0.99976	-1.00000
50	98	TROCHOPHORE	ET5.2	4	0.0	768.0	0.0	195840.0	ē	0.57622	-1.73205
50	98	TROCHOPHORE	CB2.2	3	0.0	6144.0	0.0	12576768.0		0.57721	-1.73205
51	98	GASTROPODA	TF1.5	4	400.0	4096.0	159600.0	16773120.0	0.99875	0.99988	-0.89807
51	98	GASTROPODA	TF1.7	3	235.3	320.0	55128.0	36544.0	0.99787	0.59739	-0.27892
51	98	GASTROPODA	CB2.2	3	0.0	2.0	0.0	2.0		0.70711	-1.00000
55	97	BIVALVIA	ET5.2	4	4.0	0.0		0.0			
55	97	BIVALVIA	CB2.2	3	3.0	0.0	•	0.0	•		
55	97	BIVALVIA	LE2.2	3	1.0	0.0		0.0			
55	97	BIVALVIA	CB5.2	3	200.0	0.0	39800.0	0.0	0.99750		1.00000
55	98	PELECYPODA	ET5.2	4	0.0	8.0	0.0	56.0		0.93541	-1.00000
55	98	PELECYPODA	TF2.3	4	0.0	7168.0	0.0	7332864.0		0.37778	-2.64575
87	90	GNATHOSTOMATA	TF1.7	4	0.0	3.0	0.0	0.0		0.00000	-1.73205
87	90	GNATHOSTOMATA	TF1.5	4	0.0	92.0	0.0	276.0		0.18058	-4.79583
5001	97	POLYCHAETA	TF2.3	4	250.0	0.0	62250.0	0.0	0.99800		1.00000
5001	97	POLYCHAETA	TF1.7	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
5001	97	POLYCHAETA	ET5.2	4	235.3	0.0	55128.0	0.0	0.99787		1.00000
5001	97	POLYCHAETA	CB5.2	3	1000.0	0.0	199000.0	0.0	0.44609		2.23607
5001	97	POLYCHAETA	CB2.2	3	2000.0	0.0	798000.0	0.0	0.44665		2.23607
5001	97	POLYCHAETA	TF1.5	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
5001	97	POLYCHAETA	LE2.2	3	2000.0	512.0	798000.0	130560.0	0.44665	0.70572	1.54210
5001	97	POLYCHAETA	TF1.5	3	1600.0	0.0	1278400.0	0.0	0.70666		1.41421
5001	97	POLYCHAETA	TF1.7	3	400.0	0.0	159600.0	0.0	0.99875		1.00000

5001	98	POLYCHAETA		TF1.5	4	0.0	8.0	0.0	24.0		0.61237	-1.41421
5001	98	POLYCHAETA		CB2.2	3	0.0	56.0	0.0	56.0	•	0.13363	-5.29150
6110	98	OSTRACODA		RET2.2	4	0.0	16512.0	0.0	268427136.0		0.99223	-1.00780
6110	98	OSTRACODA		CB2.2	3	0.0	4618.0	0.0	6024714.0	•	0.53151	-1.88070
6110	98	OSTRACODA		TF1.5	4	9200.0	24647.0	3670800.0	89106372.0	0.20825	0.38299	-1.60341
6110	98	OSTRACODA		TF1.5	3	14400.0	1424.0	11505600.0	460656.0	0.23555	0.47663	3.74865
6110	98	OSTRACODA		TF1.7	3	0.0	128.0	0.0	8064.0		0.70156	-1.41421
6110	98	OSTRACODA		TF1.7	4	250.0	2.0	62250.0	0.0	0.99800	0.00000	0.99198
6110	98	OSTRACODA		TF2.3	4	1200.0	14988.0	478800.0	77661580.0	0.57663	0.58798	-1.55962
6110	98	OSTRACODA		ET5.2	4	1600.0	1544.0	638400.0	391704.0	0.49937	0.40535	0.05509
6117	11	COPEPODA		TF1.7	4	800.0	0.0	319200.0	0.0	0.70622	·	1.41421
6117	11	COPEPODA		TF1.5	3	20800.0	0.0	16619200.0	0.0	0.19599		5.09902
6117	11	COPEPODA		TF1.5	4	800.0	0.0	319200.0	0.0	0.70622	•	1.41421
6117	11	COPEPODA		TF1.7	3	470.6	0.0	110256.1	0.0	0.70560		1.41421
6117	11	COPEPODA		LE2.2	3	800.0	384.0	319200.0	48768.0	0.70622	0.57509	0.68468
6117	11	COPEPODA		ET5.2	4	235.3	0.0	55128.0	0.0	0.99787	•	1.00000
6117	11	COPEPODA		CB2.2	3	1600.0	0.0	638400.0	0.0	0.49937	•	2.00000
6119	98	HARPACTICOIDA		TF1.7	3	800.0	0.0	319200.0	0.0	0.70622		1.41421
6119	98	HARPACTICOIDA		CB5.2	3	200.0	0.0	39800.0	0.0	0.99750		1.00000
6119	98	HARPACTICOIDA		TF1.5	3	12800.0	0.0	10227200.0	0.0	0.24984	·	4.00000
6119	98	HARPACTICOIDA		CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	•	1.00000
6119	98	HARPACTICOIDA		TF1.5	4	2800.0	0.0	1117200.0	0.0	0.37749		2.64575
6119	98	HARPACTICOIDA		LE2.2	3	1600.0	0.0	638400.0	0.0	0.49937		2.00000
6154	98	CUMACEA		TF1.5	3	1.0	0.0		0.0			
615301	98	MYSIDAE		CB2.2	3	2.0	0.0		0.0			
615301	98	MYSIDAE		TF1.7	3	1.0	0.0		0.0			
648933	21	CHIRONOMIDAE		TF1.5	4	21.0	0.0		0.0			
648933	21	CHIRONOMIDAE		RET2.2	4	1.0	0.0		0.0			
648933	97	CHIRONOMIDAE		TF1.5	4	5.0	0.0		0.0			
648933	97	CHIRONOMIDAE		RET2.2	4	1.0	0.0		0.0			
648933	97	CHIRONOMIDAE		TF1.7	3	1.0	0.0		0.0			
648933	97	CHIRONOMIDAE		ET5.2	4	1.0	0.0		0.0			
648933	97	CHIRONOMIDAE		TF2.3	4	6.0	0.0		0.0			
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF1.7	3	0.0	512.0	0.0	261632.0		0.99902	-1.00000
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF2.3	4	250.0	2.0	62250.0	2.0	0.99800	0.70711	0.99197
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF1.5	4	0.0	256.0	0.0	65280.0		0.99804	-1.00000
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF1.5	3	0.0	32.0	0.0	992.0	·	0.98425	-1.00000

61090103	98	SIDA	CRYSTALLINA	TF2.3	4	1.0	0.0		0.0			
61090103	98	DAPHNIA	LONGISPINA	TF1.7	3	2000.0	1536.0	798000.0	784896.0	0.44665	0.57679	0.36839
61090201	98	DAPHNIA	PULEX	RET2.2	4	250.0	0.0	62250.0	0.0	0.99800	0.37079	1.00000
61090201	98	DAPHNIA	TOLLX	CB2.2	3	800.0	0.0	319200.0	0.0	0.70622	•	1.41421
61090201	98	DAPHNIA	LONGISPINA	TF1.5	4	1450.0	2056.0	541050.0	2095128.0	0.50728	0.70402	-0.37299
61090201	98	DAPHNIA	LONGISPINA	TF2.3	4	250.0	38.0	62250.0	998.0	0.99800	0.83135	0.84106
61090201	98	DAPHNIA	LONGISPINA	TF1.5	3	9600.0	2276.0	7670400.0	675108.0	0.28849	0.83133	2.53345
61090201	98	DAPHNIA	PULEX	TF1.7	4	250.0	0.0	62250.0	0.0	0.20049		1.00000
61090301	98	BOSMINA	LONGIROSTRIS	ET5.2	4	400.0	2082.0	159600.0	4192480.0	0.99875	0.98346	-0.80603
61090301	98	BOSMINA	LONGIROSTRIS	TF1.5	3	24000.0	2082.0	19176000.0	1063776.0	0.18246	0.46712	-0.80003 <b>4.84076</b>
61090301	98	BOSMINA	LONGIROSTRIS	CB2.2	3	24000.0	512.0	0.0	261632.0	0.16240	0.40712	-1.00000
61090301	98	BOSMINA	LONGIROSTRIS	TF2.3	3 4	36400.0	39648.0	14523600.0	159366494.0	0.10470	0.99902	-0.24625
61090301	98	BOSMINA	LONGIROSTRIS	TF1.7	3	2800.0	2048.0	1117200.0	358400.0	0.37749	0.29232	0.61805
61090301	98	BOSMINA	LONGIROSTRIS	TF1.7	4	250.0	771.0	62250.0	294144.0	0.99800	0.70344	-0.87147
61090301	98	BOSMINA	LONGIROSTRIS	TF1.5	4	14000.0	39945.0	5586000.0	110584856.0	0.16882	0.26326	-2.40660
61090301	98	BOSMINA	LONGIROSTRIS	RET2.2	4	0.0	64.0	0.0	4032.0		0.99216	-1.00000
61090502	98	PODON	POLYPHEMOIDES	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61090502	98	PODON	POLYPHEMOIDES	CB5.2	3	800.0	2112.0	159200.0	2097088.0	0.49875	0.68567	-0.87288
61090502	98	PODON	POLYPHEMOIDES	LE2.2	3	11600.0	12032.0	4628400.0	6738176.0	0.18546	0.21574	-0.12800
61090502	98	PODON	POLYPHEMOIDES	TF2.3	4	0.0	128.0	0.0	16256.0	•	0.99609	-1.00000
61090601	98	LEPTODORA	KINDTII	TF2.3	4	7.0	0.0		0.0			•
61090701	98	ALONA		TF2.3	4	0.0	17408.0	0.0	134724608.0		0.66677	-1.49968
61090702	98	CHYDORUS		TF1.7	3	800.0	0.0	319200.0	0.0	0.70622		1.41421
61090702	98	CHYDORUS		CB2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61090702	98	CHYDORUS		TF1.5	3	4800.0	0.0	3835200.0	0.0	0.40799		2.44949
61090702	98	CHYDORUS		TF2.3	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
61090705	98	LEYDIGIA	QUADRANGULARIS	TF2.3	4	1200.0	0.0	478800.0	0.0	0.57663		1.73205
61090805	98	ILYOCRYPTUS	SPINIFER	TF2.3	4	2000.0	0.0	798000.0	0.0	0.44665		2.23607
61090805	98	ILYOCRYPTUS	SPINIFER	TF1.7	3	2000.0	0.0	798000.0	0.0	0.44665		2.23607
61090805	98	ILYOCRYPTUS	SPINIFER	TF1.5	4	24800.0	0.0	9895200.0	0.0	0.12684		7.87401
61090805	98	ILYOCRYPTUS	SPINIFER	TF1.5	3	46400.0	0.0	37073600.0	0.0	0.13122		7.61577
61180505	12	PSEUDOCALANUS		TF1.5	4	0.0	39424.0	0.0	45835776.0		0.17173	-5.82065
61180505	12	PSEUDOCALANUS		RET2.2	4	0.0	512.0	0.0	261632.0		0.99902	-1.00000
61180505	12	PSEUDOCALANUS		TF1.7	4	0.0	33792.0	0.0	34569216.0		0.17399	-5.74456
61180505	12	PSEUDOCALANUS		CB5.2	3	200.0	384.0	39800.0	48768.0	0.99750	0.57509	-0.61624
61180505	12	PSEUDOCALANUS		CB2.2	3	0.0	46592.0	0.0	74271232.0		0.18497	-5.40462
61180505	12	PSEUDOCALANUS		ET5.2	4	0.0	2048.0	0.0	4192256.0		0.99976	-1.00000
61180505	12	PSEUDOCALANUS		TF2.3	4	0.0	183296.0	0.0	1414870016.0	-	0.20521	-4.87267

61180505	98	PSEUDOCALANUS		TF1.5	3	0.0	4096.0	0.0	8384512.0		0.70693	-1.41421
61180505	98	PSEUDOCALANUS		TF1.7	3	0.0	15489.0	0.0	7406464.0		0.17570	-5.68544
61180505	98	PSEUDOCALANUS		TF1.5	4	0.0	63.0	0.0	94.0		0.15389	-5.02795
61180505	98	PSEUDOCALANUS		TF2.3	4	0.0	2586.0	0.0	206490.0		0.17572	-5.65557
61180505	98	PSEUDOCALANUS		RET2.2	4	0.0	32768.0	0.0	1073709056.0		0.99998	-1.00000
61180505	98	PSEUDOCALANUS		TF1.7	4	0.0	57871.0	0.0	107224576.0		0.17893	-5.58723
61180505	98	PSEUDOCALANUS		LE2.2	3	0.0	2048.0	0.0	2095104.0		0.70676	-1.41421
61180505	98	PSEUDOCALANUS		CB5.2	3	2400.0	2752.0	477600.0	1090880.0	0.28795	0.37952	-0.28060
61181701	12	CENTROPAGES		TF2.3	4	500.0	0.0	124500.0	0.0	0.70569		1.41421
61181701	12	CENTROPAGES		CB5.2	3	6200.0	736.0	1233800.0	84256.0	0.17916	0.39439	4.74683
61181701	12	CENTROPAGES		LE2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61181701	98	CENTROPAGES	TYPICUS	CB5.2	3	5408.0	2240.0	1074600.0	370496.0	0.19168	0.27173	2.62839
61181701	98	CENTROPAGES	HAMATUS	LE2.2	3	0.0	2.0	0.0	2.0		0.70711	-1.00000
61181801	12	DIAPTOMUS		TF1.5	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
61181801	12	DIAPTOMUS		TF1.5	3	1600.0	384.0	1278400.0	48768.0	0.70666	0.57509	1.05474
61181801	12	DIAPTOMUS		TF1.7	4	250.0	0.0	62250.0	0.0	0.99800		1.00000
61181801	12	DIAPTOMUS		TF1.7	3	0.0	3008.0	0.0	877632.0		0.31144	-3.20537
61181801	12	DIAPTOMUS		TF2.3	4	250.0	0.0	62250.0	0.0	0.99800		1.00000
61181801	98	DIAPTOMUS		TF2.3	4	250.0	0.0	62250.0	0.0	0.99800		1.00000
61181801	98	DIAPTOMUS		CB5.2	3	0.0	1024.0	0.0	523264.0		0.70642	-1.41421
61181801	98	DIAPTOMUS		LE2.2	3	0.0	5512.0	0.0	1682056.0		0.23529	-4.24305
61181801	98	DIAPTOMUS		TF1.5	3	1000.0	32.0	499000.0	992.0	0.70640	0.98425	1.36756
61181801	98	DIAPTOMUS		TF1.7	4	1.0	0.0		0.0			
61181801	98	DIAPTOMUS		TF1.7	3	0.0	1026.0	0.0	1047552.0		0.99756	-1.00195
61181902	12	PSEUDODIAPTOMUS	CORONATUS	CB5.2	3	1800.0	0.0	358200.0	0.0	0.33250		3.00000
61181902	98	PSEUDODIAPTOMUS	CORONATUS	CB5.2	3	2000.0	0.0	398000.0	0.0	0.31544		3.16228
61182002	12	EURYTEMORA		TF1.7	3	198000.0	34305.0	395802000.0	31685120.0	0.10048	0.16409	7.91510
61182002	12	EURYTEMORA		TF1.7	4	1096000.0	31746.0	4382904000.0	38765568.0	0.06040	0.19613	16.00280
61182002	12	EURYTEMORA		TF2.3	4	408000.0	462864.0	1631592000.0	7270166542.0	0.09900	0.18421	-0.58150
61182002	12	EURYTEMORA		ET5.2	4	550000.0	139640.0	1099450000.0	247327248.0	0.06029	0.11262	11.17910
61182002	12	EURYTEMORA		CB2.2	3	334000.0	155474.0	667666000.0	289370322.0	0.07736	0.10941	5.76930
61182002	12	EURYTEMORA		CB5.2	3	800.0	64.0	159200.0	4032.0	0.49875	0.99216	1.81690
61182002	12	EURYTEMORA		RET2.2	4	2088000.0	563149.0	8349912000.0	7455168588.0	0.04376	0.15332	12.12810
61182002	12	EURYTEMORA		LE2.2	3	170000.0	56353.0	339830000.0	53683230.0	0.10844	0.13002	5.72730
61182002	12	EURYTEMORA		TF1.5	3	608000.0	30560.0	2431392000.0	13759648.0	0.08110	0.12138	11.67610
61182002	12	EURYTEMORA		TF1.5	4	342000.0	59648.0	683658000.0	132126464.0	0.07645	0.19271	9.88320
61182002	98	EURYTEMORA	AFFINIS	TF2.3	4	78400.0	68376.0	62641600.0	134509846.0	0.10095	0.16962	0.71360

61182002	98	EURYTEMORA	AFFINIS	ET5.2	4	210000.0	49749.0	419790000.0	100621176.0	0.09757	0.20163	7.02290
61182002	98	EURYTEMORA	AFFINIS	CB5.2	3	400.0	256.0	79600.0	16128.0	0.70534	0.49608	0.46380
61182002	98	EURYTEMORA	AFFINIS	TF1.7	4	240000.0	88623.0	959760000.0	145925632.0	0.12908	0.13631	4.55180
61182002	98	EURYTEMORA	AFFINIS	TF1.7	3	110400.0	78860.0	88209600.0	160353280.0	0.08507	0.16058	1.99980
61182002	98	EURYTEMORA	AFFINIS	CB2.2	3	182000.0	177286.0	363818000.0	680873286.0	0.10480	0.14718	0.14580
61182002	98	EURYTEMORA	AFFINIS	LE2.2	3	27200.0	2072.0	10852800.0	4192276.0	0.12112	0.98818	6.47200
61182002	98	EURYTEMORA	AFFINIS	TF1.5	4	164000.0	115002.0	327836000.0	439828270.0	0.11040	0.18236	1.76810
61182002	98	EURYTEMORA	AFFINIS	RET2.2	4	424000.0	929842.0	1695576000.0	28060012590.0	0.09712	0.18015	-2.93240
61182002	98	EURYTEMORA	AFFINIS	TF1.5	3	320000.0*	47184.0	511680000.0	84625648.0	0.07069	0.19496	11.16870
61182003	12	TEMORA	TURBINATA	CB5.2	3	4.0	0.0		0.0			
61182003	98	TEMORA	LONGICORNIS	CB5.2	3	0.0	96.0	0.0	2976.0		0.56826	-1.73205
61182901	12	ACARTIA		RET2.2	4	400.0	1408.0	159600.0	88704.0	0.99875	0.21153	-2.01555
61182901	12	ACARTIA		TF1.7	4	15600.0	2816.0	6224400.0	357632.0	0.15993	0.21237	4.97600
61182901	12	ACARTIA	TONSA	TF1.7	3	235.3	0.0	55128.0	0.0	0.99787		1.00000
61182901	12	ACARTIA		LE2.2	3	34800.0	11776.0	13885200.0	10080768.0	0.10708	0.26962	4.69853
61182901	12	ACARTIA	TONSA	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61182901	12	ACARTIA		ET5.2	4	1600.0	11024.0	638400.0	2807280.0	0.49937	0.15199	-5.06762
61182901	12	ACARTIA		CB5.2	3	16400.0	3872.0	3263600.0	402656.0	0.11016	0.16388	6.52489
61182901	98	ACARTIA	TONSA	CB5.2	3	14800.0	10880.0	2945200.0	5252480.0	0.11596	0.21065	1.36698
61182901	98	ACARTIA	TONSA	ET5.2	4	8800.0	5520.0	3511200.0	1371888.0	0.21293	0.21219	1.48215
61182901	98	ACARTIA	TONSA	CB2.2	3	2400.0	0.0	957600.0	0.0	0.40774		2.44949
61182901	98	ACARTIA	TONSA	LE2.2	3	17200.0	2048.0	6862800.0	2095104.0	0.15231	0.70676	5.05709
61182901	98	ACARTIA	TONSA	RET2.2	4	28000.0	67394.0	11172000.0	2147535042.0	0.11937	0.68762	-0.84786
61182901	98	ACARTIA	TONSA	TF1.7	4	27200.0	13825.0	10852800.0	8112640.0	0.12112	0.20602	3.06791
61182901	98	ACARTIA	TONSA	TF2.3	4	0.0	1031.0	0.0	1047558.0		0.99273	-1.00683
61190502	98	CANUELLA	ELONGATA	TF2.3	4	0.0	32.0	0.0	992.0		0.98425	-1.00000
61190502	98	CANUELLA	ELONGATA	TF1.7	3	0.0	192.0	0.0	12096.0		0.57282	-1.73205
61190502	98	CANUELLA	ELONGATA	TF1.7	4	250.0	2560.0	62250.0	1308160.0	0.99800	0.44678	-1.97125
61190502	98	CANUELLA	ELONGATA	TF1.5	4	20000.0	25289.0	7980000.0	83883334.0	0.14124	0.36216	-0.55169
61190502	98	CANUELLA	ELONGATA	TF1.5	3	0.0	3008.0	0.0	4537408.0		0.70815	-1.41166
61191401	98	EUTERPINA	ACUTIFRONS	LE2.2	3	0.0	257.0	0.0	32512.0		0.70160	-1.41972
61200801	12	HALICYCLOPS		TF1.5	4	0.0	16896.0	0.0	8633856.0		0.17391	-5.74456
61200801	12	HALICYCLOPS		LE2.2	3	0.0	768.0	0.0	195840.0		0.57622	-1.73205
61200801	12	HALICYCLOPS		CB5.2	3	0.0	64.0	0.0	1984.0		0.69597	-1.41421
61200801	98	HALICYCLOPS		CB5.2	3	0.0	128.0	0.0	16256.0		0.99609	-1.00000
61200801	98	HALICYCLOPS		TF1.5	4	36000.0	10241.0	14364000.0	20961280.0	0.10528	0.44706	4.33114
61200801	98	HALICYCLOPS		TF1.7	4	0.0	384.0	0.0	48768.0		0.57509	-1.73205

61200801	98	HALICYCLOPS		TF2.3	4	0.0	26752.0	0.0	202364800.0		0.53175	-1.88044
61200801	98	HALICYCLOPS		TF1.5	3	19200.0	7450.0	15340800.0	4875258.0	0.20400	0.29638	2.61158
61200802	12	CYCLOPS		TF2.3	4	1200.0	0.0	478800.0	0.0	0.57663		1.73205
61200802	12	CYCLOPS		TF1.7	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61200802	12	CYCLOPS		CB2.2	3	705.9	0.0	165384.1	0.0	0.57612		1.73205
61200802	12	CYCLOPS		TF1.5	3	3200.0	0.0	2556800.0	0.0	0.49969		2.00000
61200802	12	CYCLOPS		TF1.5	4	2000.0	0.0	798000.0	0.0	0.44665		2.23607
61200802	12	CYCLOPS		TF1.7	4	750.0	0.0	186750.0	0.0	0.57619		1.73205
61200802	98	CYCLOPS	BICUSPIDATUS	RET2.2	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
61200802	98	CYCLOPS	BICUSPIDATUS	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61200802	98	CYCLOPS	VERNALIS	TF1.5	3	1600.0	0.0	1278400.0	0.0	0.70666	•	1.41421
61200802	98	CYCLOPS	BICUSPIDATUS	TF1.5	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
61200803	12	MESOCYCLOPS		CB2.2	3	0.0	1792.0	0.0	456960.0		0.37723	-2.64575
61200803	12	MESOCYCLOPS	EDAX	TF2.3	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
61200803	12	MESOCYCLOPS		TF1.5	4	0.0	1024.0	0.0	523264.0		0.70642	-1.41421
61200803	98	MESOCYCLOPS	EDAX	TF2.3	4	2.0	3202.0		4731778.0		0.67935	·
61200803	98	MESOCYCLOPS	EDAX	TF1.5	4	0.0	1026.0	0.0	523266.0		0.70504	-1.41697
61200804	98	EUCYCLOPS	SPERATUS	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61200804	98	EUCYCLOPS	SPERATUS	TF1.5	4	250.0	0.0	62250.0	0.0	0.99800	٠	1.00000
61200804	98	EUCYCLOPS	AGILIS	TF2.3	4	800.0	0.0	319200.0	0.0	0.70622		1.41421
61200807	98	TROPOCYCLOPS	PRASINUS	TF1.7	3	400.0	0.0	159600.0	0.0	0.99875	•	1.00000
61200901	12	OITHONA		CB5.2	3	0.0	32.0	0.0	992.0		0.98425	-1.00000
61200901	98	OITHONA		TF1.7	4	0.0	2048.0	0.0	2095104.0		0.70676	-1.41421
61200901	98	OITHONA	COLCARVA	CB5.2	3	0.008	0.0	159200.0	0.0	0.49875		2.00000
61200901	98	OITHONA		TF2.3	4	0.0	34049.0	0.0	537918208.0		0.68117	-1.46802
613402	11	BALANIDAE		CB2.2	3	1600.0	0.0	638400.0	0.0	0.49937		2.00000
613402	11	BALANIDAE		CB5.2	3	82000.0	0.0	81918000.0	0.0	0.11038		9.05539
613402	11	BALANIDAE		LE2.2	3	192000.0	0.0	383808000.0	0.0	0.10204		9.79796
613402	11	BALANIDAE		TF1.5	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
613402	11	BALANIDAE		TF1.7	4	7200.0	0.0	2872800.0	0.0	0.23541		4.24264
613402	11	BALANIDAE		ET5.2	4	4400.0	0.0	1755600.0	0.0	0.30113		3.31662
613402	17	BALANIDAE		ET5.2	4	400.0	0.0	159600.0	0.0	0.99875		1.00000
613402	17	BALANIDAE		CB5.2	3	5400.0	0.0	1074600.0	0.0	0.19197		5.19615
613402	17	BALANIDAE		CB2.2	3	400.0	0.0	159600.0	0.0	0.99875		1.00000
61340201	11	BALANUS		ET5.2	4	0.0	1032.0	0.0	261176.0		0.49521	-2.01538
61340201	11	BALANUS		LE2.2	3	0.0	77842.0	0.0	132042768.0		0.14762	-6.77218
61340201	11	BALANUS		RET2.2	4	0.0	8192.0	0.0	67100672.0		0.99994	-1.00000

61340201	11	BALANUS		CB5.2	3	0.0	32321.0	0.0	26717632.0		0.15992	-6.24918
61340201	11	BALANUS		TF1.7	3	0.0	1.0	0.0	0.0		0.00000	-1.00000
61340201	11	BALANUS		TF1.7	4	0.0	7040.0	0.0	2860160.0		0.24023	-4.15761
61340201	17	BALANUS		TF2.3	4	0.0	3078.0	0.0	3142662.0	•	0.57594	-1.73543
61340201	17	BALANUS		TF1.7	3	0.0	1.0	0.0	0.0		0.00000	-1.00000
61340201	17	BALANUS		CB5.2	3	0.0	3392.0	0.0	1581760.0		0.37078	-2.69414
61340201	17	BALANUS		CB2.2	3	0.0	2048.0	0.0	4192256.0		0.99976	-1.00000
61340201	17	BALANUS		TF1.5	4	0.0	768.0	0.0	326912.0		0.74448	-1.34164
61340201	17	BALANUS		RET2.2	4	0.0	193.0	0.0	12096.0		0.56985	-1.74100
61340201	17	BALANUS		LE2.2	3	0.0	1024.0	0.0	1047552.0		0.99951	-1.00000
61530115	93	NEOMYSIS	AMERICANA	TF1.7	3	0.0	2.0	0.0	0.0		0.00000	-1.41421
61530115	93	NEOMYSIS	AMERICANA	RET2.2	4	0.0	17.0	0.0	12.0		0.20377	-3.15682
61530115	93	NEOMYSIS	AMERICANA	CB2.2	3	0.0	4302.0	0.0	16785230.0		0.95234	-1.04991
61530115	98	NEOMYSIS	AMERICANA	CB2.2	3	75.0	26.0		26.0		0.19612	
61530115	98	NEOMYSIS	AMERICANA	CB5.2	3	11.0	1.0		0.0		0.00000	•
61530115	98	NEOMYSIS	AMERICANA	RET2.2	4	56.0	31.0		26.0		0.16448	
61530115	98	NEOMYSIS	AMERICANA	TF1.7	3	17.0	7.0		0.0		0.00000	
61530115	98	NEOMYSIS	AMERICANA	ET5.2	4	70.0	48.0		408.0		0.42081	
61530121	98	MYSIDOPSIS	BIGELOWI	CB2.2	3	2.0	0.0		0.0		•	•
61540508	98	OXYUROSTYLIS	SMITHI	CB5.2	3	2.0	0.0		0.0			
61691502	98	COROPHIUM	LACUSTRE	TF1.5	3	2.0	0.0		0.0			
61691502	98	COROPHIUM	LACUSTRE	TF1.5	4	12.0	0.0		0.0			
61691502	98	COROPHIUM	LACUSTRE	TF1.7	3	6.0	0.0		0.0			
61692107	98	GAMMARUS		CB2.2	3	11.0	0.0		0.0			
61692107	98	GAMMARUS		RET2.2	4	0.0	2.0	0.0	2.0		0.70711	-1.00000
61692107	98	GAMMARUS		TF1.5	3	80.0	0.0		0.0			
61692107	98	GAMMARUS		TF1.5	4	117.0	4363.0		16781080.0		0.93891	
61692107	98	GAMMARUS		TF1.7	3	52.0	2.0		0.0		0.00000	
61692107	98	GAMMARUS		TF2.3	4	13.0	8.0		8.0		0.35355	
61693708	98	MONOCULODES		CB2.2	3	19.0	0.0		0.0			
61693708	98	MONOCULODES		CB5.2	3	1.0	0.0		0.0		•	•
61693708	98	MONOCULODES		TF1.5	3	10.0	0.0		0.0			
61693708	98	MONOCULODES		TF1.5	4	23.0	0.0		0.0			
61693708	98	MONOCULODES		TF1.7	3	1.0	0.0		0.0			
61792201	31	CRANGON	SEPTEMSPINOSA	CB5.2	3	0.0	2.0	0.0	0.0		0.00000	-1.41421
61792201	31	CRANGON	SEPTEMSPINOSA	LE2.2	3	0.0	2.0	0.0	2.0		0.70711	-1.00000
61792201	31	CRANGON	SEPTEMSPINOSA	TF1.7	3	0.0	1.0	0.0	0.0		0.00000	-1.00000

61792201	98	CRANGON	SEPTEMSPINOSA	CB5.2	3	24.0	0.0		0.0		
61792201	98	CRANGON	SEPTEMSPINOSA	LE2.2	3	3.0	0.0	•	0.0		
64890502	98	CHAOBORUS	PUNCTIPENNIS	TF1.5	3	3.0	0.0		0.0		
64890502	98	CHAOBORUS	PUNCTIPENNIS	CB2.2	3	2.0	0.0		0.0		
64890502	98	CHAOBORUS	PUNCTIPENNIS	TF1.5	4	0.0	32.0	0.0	992.0	0.98425	-1.00000

<sup>\*</sup> The corrected number is 1,216,000.

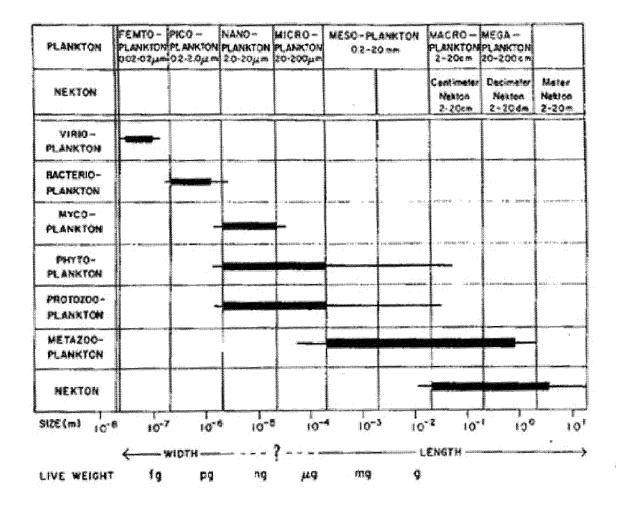
## **Appendix E: Zooplankton Classifications**

Classifying the plankton on the basis of characteristics such as structural organization, reproduction and growth rate, and mode of nutrition "provides the ecologist with a rational system for putting the components of the pelagic ecosystem into compartments and then equating these with plankton size fractions and methods for estimating their populations" (Sieburt et al. 1978). The size fractions are at times an artificial division of the compartments and functional groups that is forced on the ecologist by the mesh sizes of the nets used to collect plankton (Sieburt et al. 1978). Size, however, can prove to be a useful means of dividing the plankton because it "is a decisive factor in governing growth rate and doubling time of plankton organisms. Since within the pelagic food web most predators swallow their prey organisms undivided, body size also determines food-chain relationships" (Harris et al. 2000).

The "metazooplankton" were identified by Sieburt et al. (1978) as a compartment of the pelagic ecosystem consisting of "multicellular ingesting forms." They typically span a length range of approximately 200 microns to 100 centimeters, but immature forms can be as small as 50 microns and some individuals reach 200 cm in length (Figure E-1). Sieburth et al. (1978) divided the metazooplankton into three functional groups and equated them with three size fractions: the "mesoplankton" (200 micron - 20 mm), the "macroplankton" (20 mm - 20 cm), and the "megaplankton" (20 - 200 cm). Mesozooplankton in ocean settings consist mainly of copepods whose copepodite and adult sizes almost exactly match the length limits of the size fraction. This functional group also includes cladocera, meroplanktic larvae, small hydromedusae and ctenophores, chaetognaths, appendicularians, doliolids, ostracods, and fish eggs and small fish larvae (Sieburth et al. 1987, Harris et al. 2000, Day et al. 1989). The macrozooplankton are generally active swimmers and carnivorous. They include large crustaceans such as hyperiid amphipods, mysids, and euphausiids, the larger ctenophores, hydromedusae, and scyphomedusae, and the larger fish larvae. Megazooplankton are the still larger, drifting forms such as enidarians and pelagic tunicates. This classification scheme is widely used and was recently affirmed by International Council for the Exploration of the Sea, or ICES (Harris et al. 2000). In order to catch representative samples of the three functional groups, plankton collection methodology is usually tailored to the population characteristics of each size fraction in the body of water being surveyed.

The protozooplankton are another plankton compartment in the pelagic ecosystem and consist of the nanozooplankton and the microzooplankton functional groups (Sieburth et al. 1978). Nanozooplankton are the apochlorotic (heterotrophic) flagellates and amoeboid forms, and are equated to the smaller, 2 - 20 micron size fraction. Microzooplankton are the rotifers, ciliates, and the eggs and early life stages of crustacean plankton and meroplanktic larvae, and they are equated to the 20 - 200 micron "microplankton" size fraction although some ciliates are smaller than 20 microns (Harris et al. 2000).

Figure E-1. Size spectrum of different taxonomic-trophic compartments of plankton including the size range of nekton (from Sieburth et al. 1978).



# Appendix F: Tracking Sheet for Reviews of the April 2000 Draft Report on the Mesozooplankton Split Sample Study

June 6, 2000

Recommendations and comments received from reviewers by May 17, 2000 were incorporated into this tracking sheet. Text referenced in the recommendations and comments was copied and highlighted in the tracking sheet. Minor changes, and suggested spelling and punctuation corrections, were simply fixed and are not listed here. Specific change(s) to the report were proposed in response to the recommendations and comments. These proposed changes were reviewed, somewhat modified, and approved by the Chesapeake Bay Program Monitoring Subcommittee coordinator and the program managers in the Virginia Department of Environmental Quality and the Maryland Department of Natural Resources, and then implemented. They are listed as "action(s)" in this tracking sheet.

#### Report structure

Recommendation: The report as it is now should be reorganized to emphasize the results of the last 20 split samples. The results of the first round are of some historical interest but should not be considered when making judgements about the CVS versus the stempel pipette method. Some of the discussion in the first round should be saved. However, the results of the entire 20 samples from the second round are the results that really count and these should be considered in toto as the main part of the report. (Kent Carpenter) Comments:

- I find the report hard to follow since it is basically a compendium of the various minutes of the zooplankton conference and various conference calls. (Kent Carpenter)
- Most of the background information in the first paragraph [page 6] is irrelevant to the results and conclusion. Please state the total number of samples in Round 2. It's a little confusing you have to read this section carefully to know if 20 or 25 samples were done. (Mary Ellen Ley)
- If you haven't done so already, combine the discussion, results and tables from the 1<sup>st</sup> five with the 1<sup>st</sup> ten. It would make more sense to do data analyses on all 20 samples instead of the 1<sup>st</sup> ten and 2<sup>nd</sup> ten. (Mary Ellen Ley)

Action: Text of the Round 1, "Plankton Summit," and Round 2 sections was condensed. The various subsections in Round 2 were left separate because changes (e.g. counting methods, species identifications) occurred between the first five, the second five and the last ten split sample counts. The Round 2 text, however, was reworked to try and make it clearer. A Discussion section was added to further discuss issues raised by all reviewers and summarize key points. This tracking sheet was created.

<u>Definition of "mesozooplankton" and extrusion/clogging problems with towed plankton nets</u>

<u>Recommendation</u>: The short comings of sampling and counting zooplankton smaller than 200 microns was discussed at length during the split sampling program and yet nowhere in this report is it mentioned. I recommend that the argument be included in order to show that all aspects of the mesozooplankton monitoring was thoroughly discussed. (Kent Carpenter) Comments:

- The definition of mesozooplankton are those heterotrophic organisms in the size range of 200 microns to 2000 microns. This classification is based on taxon and trophic considerations (ICES, 2000). Nowhere in the ICES manual (or anywhere else in the published scientific literature) as far as I can tell does it state that the definition of mesozooplankton is defined as the plankton that is retained on a 200 micron sampling net. The plankton that is retained on a 200 micron sampling net is closely approximated as mesozooplankton for OCEANOGRAPHIC purposes because the density of zooplankton is relatively small and detritus is not a clogging factor in the open ocean. However, under ESTUARINE conditions, productivity and hence densities of zooplankton are much higher and there is often considerable detritus. Both these factors effectively reduce the mesh size of the plankton net due to clogging. "It is fairly obvious that as clogging increases the mesh size will decrease, with a corresponding effect on mesh selection. Clogging is greater with fine meshes and in highly productive waters." (UNESCO, 1968). Therefore, a large proportion of individuals smaller than 200 microns can be collected on a 200 micron mesh net and subsequently counted as mesozooplankton under estuarine conditions when they are in fact, microzooplankton. Furthermore, the density of zooplankton and detritus in an estuarine condition are highly variable and very patchy and therefore the effective mesh size of a 200 micron plankton net will vary depending on conditions. This means that this zooplankton smaller than 200 microns (microzooplankton) cannot be reliably or accurately measured, especially under estuarine conditions. Furthermore, even in waters that are relatively oligotrophic, extrusion of zooplankton at normal towing speeds reduces reliability of sampling organisms larger than the mesh size of the plankton net (UNESCO, 1968). Because of this, "it is advisable to use a net with a mesh size of about 75% of the width of the smallest organisms to be sampled." (Omori and Ikeda, 1992). In other words, to reliably sample zooplankton even at the 200 micron size, a plankton mesh size of 150 microns would be required. These limitations to reliable sampling of zooplankton less than 200 microns, given the mesh size of the sampling net are the reasons the Virginia subsampling methodology used a lower limit of 200 microns in their CVS method. (Kent Carpenter)
- I disagree with Kent's comment that "nowhere in the ICES Manual (or anywhere else in the published scientific literature) as far as I can tell does it state that the definition of mesozooplankton is defined as the plankton that is retained on a 200 micron sampling net." On page 320 of the ICES manual (Harris et al. 2000) it is stated that, "Meso- and macrozooplankton are defined as being retained on meshes of 200um and 2000um, respectively......" The net size is 200u and should remain that size. Everything retained in that net needs to be retained prior to any kind of enumeration. This is what we mean by the term mesozooplankton. Therefore, any field or laboratory handling (sieving) after collection of the sample should be filtered through mesh sizes smaller than 200u. Zooplankton programs at VIMS, City University of New York, and Lamont-Doughery at Columbia .....CBI, ANS, VIMS and Versar.....all followed this procedure. We should include this as a recommended procedure for handling of samples. Rinsing the sampling thoroughly is also extremely important to ensure that all organisms caught in the sample find their way into the cod end. Otherwise, lots of organisms, especially the smaller ones, are likely to remain stuck on the meshes, won't ever be enumerated, and an underestimate of density will result. The goal of the shipboard and laboratory handling is to assure that

everything captured in the net somehow is represented in a density estimate. I can't stress this enough. (Fred Jacobs)

• Reply to some of Kent's points (Claire Buchanan):

In its Introduction (pages 1-13) and elsewhere (e.g. pg 320 mentioned by Fred Jacobs), the ICES Manual offers general definitions of the various zooplankton types, including mesozooplankton, and discusses the need for and the uses of zooplankton size classifications. This information is largely derived from Sieburth et al. (1978) and earlier authors. Sieburth et al. (1978) point out that zooplankton as a whole span a wide size spectrum (six orders of magnitude) which necessitates grouping them into size fractions that can be effectively collected. The upper and lower limits chosen for each size fraction were selected so that they encompass the bulk of an individual zooplankton category (for example, "mesozooplankton"). Since nets were - and still are - the primary means of collected zooplankton greater than 20 micron, this meant that plankton nets with mesh openings equal to the lower size limit should collect the bulk of an individual zooplankton category when towed correctly in the water. A size range of 200 micron - 20 mm (body length) was selected for the mesozooplankton even though immature individuals of some species are smaller than 200 microns and hence not adequately sampled by the 200 micron mesh plankton net. Microzooplankton taxa (e.g. copepod nauplii, rotifers) are indeed caught in the 200

Microzooplankton taxa (e.g. copepod nauplii, rotifers) are indeed caught in the 200 micron plankton nets and counted by the mesozooplankton monitoring programs, however these taxa are not included in calculations of bay-wide mesozooplankton indicators.

Clogging and extrusion are important issues to consider when using plankton nets to collect mesozooplankton as well as while handling samples on ship-board and in the laboratory. However, clogging/extrusion problems experienced with *towed plankton nets* are not identical to clogging/extrusion problems experienced with *sieves*. The "limitations to reliable sampling of zooplankton less than 200 microns" with a 200 micron towed plankton net in the field are not valid reasons for using a 200 micron sieve on the bottom of the CVS method stack of sieves in the laboratory.

<u>Action</u>: Appendix E (definitions of mesozooplankton and microzooplankton) inserted. Paragraphs on "what are mesozooplankton?" and counting mesozooplankton smaller than 200 micron inserted in new Discussion section of report.

#### General comments on Draft Executive Summary

- I believe the Executive Summary is well written and gets across the major points. (Fred Jacobs)
- Add sentence that states that the [CVS method] patch didn't work and the methods do not produce comparable data. Hence, need to use a single method for baywide determinations. (Mary Ellen Ley)

Action: Added the sentence "A single method needs to be selected and implemented because the modified laboratory methods of the two programs still do not produce comparable results."

# Text about taxa lost by CVS sieving protocol and resulting undercount

"The Maryland and Virginia mesozooplankton monitoring programs implemented modifications to their respective laboratory counting protocols in 1998 in order to better estimate species richness in Maryland and to eliminate large sieving losses of smaller taxa in Virginia." (Draft Executive Summary, pg 1, first paragraph.)

"However, the "new" ODU method still produces split sample results with significantly lower total counts than those of Versar. It appears to selectively undercount key taxa, particularly the immature (copepodite) life stage of calanoid copepods, a common and frequently dominant taxonomic group." (Draft Executive Summary, page 1, first paragraph.)

<u>Recommendation</u>: Replace "eliminate...." with "add coverage of zooplankton smaller than 200 microns." (Kent Carpenter)

<u>Recommendations</u>: Please replace "It appears..." with: "It consistently counts less of certain" and "dominant taxonomic group largely occurring in the below 200 micron size range." (Kent Carpenter)

- The so called sieving losses of smaller taxa was built into the design of the Virginia CVS subsampling method because the mesh size of the sampling net in the field is 200 microns. This 200 micron mesh size was chosen as the lowest mesh size of the Virginia subsampling method because the intent was to monitor MESOZOOPLANKTON. (Kent Carpenter)
- While small taxa can be caught length-wise on the sieve, body width is the critical dimension that determines retention. The ICES Manual (Harris et al. 2000), the earlier IBP Handbook No 17 (Edmondson and Winberg 1971), and other methodology papers recommend using sieves with mesh openings that are less than the length or width of the smallest individuals the investigator wants to retain. The "old" CVS method has employed five sieves since the start of the Virginia mesozooplankton monitoring program: 2000, 850, 600, 300, and 200 microns. None are smaller than 200 microns. Comparisons of counts obtained with the "new" CVS method and the "old" CVS method show that total counts increased significantly when smaller sieves were included in the "new" CVS protocol. This result demonstrates that the "old" CVS method undercounted Virginia mesozooplankton samples, and supports the statement in question. (Claire Buchanan)
- Reply to Kent's recommendation to change wording to "dominant taxonomic group largely occurring in the below 200 micron size range...." Length-width information presented in the report (new Table 11) shows that the lengths of adults and copepodites of *Acartia tonsa* and *Eurytemora affinis*, two of the dominant calanoid copepod taxa, do not largely occur "in the below 200 micron size range." Likewise, the lengths of immature and adult *Bosmina longirostris*, a seasonally dominant cladoceran species, does not largely occur in the <200 micron range. (Claire Buchanan)
- The IBP Manual No. 17 (Edmondson and Winberg, 1971) makes the following recommendation (pg. 136-137): "If subsampling with a pipette is necessary, one should show that a sorting bias is not introduced. This can be done by fractionating a whole sample and counting subsamples from the beginning and end of the series; there should be no significant tendency for one kine of animal (the largest or smallest) to be in the subsamples taken first or last. Subsampling should be practiced and subsamples counted

until the operator is able to show that the aliquotes counts are randomly distributed." (Mary Ellen Ley)

• See also Sample handling in CVS method below

Actions: Changed text to "...eliminate laboratory sieving losses of small mesozooplankton taxa and life stages in Virginia." Changed text to "However, the "new" ODU method still produces significantly lower total counts than the Versar method. The method consistently counts less of certain taxa, particularly the immature (copepodite) life stage of calanoid copepods which are a common and frequently dominant taxonomic group."

## Clumping in sample jars

<u>Recommendation</u>: Include the following text in the executive summary: "It is not clear if the Versar method overcounts these taxa because of potential clumping in their subsampling method or if the ODU method somehow undercounts these taxa." (Kent Carpenter) Comments:

- It is well know that clumping can occur in plankton samples (e.g. Longhurst and Seibert, 1967) and that zooplankton have different densities and will suspend in fluids differently depending on animal density and shape. For example, the ICES manual (p 151) when discussing enumerating techniques states "cladocerans, tend to float in the surface film." These differences could affect distribution of zooplankton even in a sample that is being mixed prior to subsampling with a stempel pipette. Since the possibility of clumping while subsampling exists for the stempel pipette and there is no evidence in the data that suggests the CVS method somehow eliminates taxa selectively, it should not be assumed that the difference in the observed abundances is due to an inadequacy of the CVS method. It could just as well be due to bias in the stempel pipette method employed by VERSAR. (Kent Carpenter)
- There does not appear to be a problem with clumping in the Stempel pipette using the Versar method as far as we can tell. Versar applies a methodology to ensure homogeneity in the sample prior to subsampling with the pipette. Early in the program Versar conducted lab counts using 3 sample replicate subsamples of the same volume. The results indicated that sample counts were usually with 5% of each other and almost always within 10%. Willie can probably dig up these old data sheets we if need to. While anything is possible, I do not think there is any basis to change the text as Kent suggests on Page 2, where he wants to introduce clumping as a potential source of error for the Versar method. (Fred Jacobs)
- Section 2.1.8 of the IBP Manual (pag 137) describes in general terms a procedure to check sampling bias. (Mary Ellen Ley)

Action: None

# Sample handling in CVS method

#### Comments:

• The motorized siever used at ODU for the CVS method is an accurate and reliable machine with minimal losses of mesozooplankton. There is an impression that has been circulating that the motorized siever is overly destructive to zooplankton during its operation and that zooplankton are typically lost in the process of sieving. True, the motorized siever does make noise, and does shake in order to facilitate the sieving

process. However, the allegations that it is overly destructive to zooplankton and unreliable are unfounded. There is no data or casual observation that supports this idea. We have placed a passive 45 micron sieve below the 63 micron passive sieve that collects all discharge from the motorized siever and find only the smallest organisms that would be expected to pass through these sieves. There is no evidence for the destructive nature of the motorized siever. The methodology I propose for the next round of split samples should test this. (Kent Carpenter)

- Standard Methods (APHA, 1995) says about the Folsom Splitter: "Exercise care to provide unbiased splits. Even when using the Folsom splitter unbiased subsamples cannot be unquestioningly assumed (McEwen et al. 1954); therefore, count animals in several subsamples from the same sample to verify that the splitter is unbiased and to determine the sampling error introduced by using it." (Mary Ellen Ley)
- Question to Fred and William: Sieburth et al (1978) make an interesting observation about how long (90u), thin (1.5u) bacteria get through a 3u millipore filter which they apparently do well (pg 1261): "...as water flows through small screens and perforated membranes, streamlines are apparently formed on a microscale, inwhich the smaller particles line up longitudinally so that only their widths affects retention." I've seen something like this while watching preserved copepods get sucked up into a pipette. Considering the water flow inside the stacked sieves of the CVS method, this seem like a likely hypothesis to explain why the long, thin mesozooplankton taxa (e.g. copepodites) get through small meshes and why George isn't seeing many broken zooplankton parts in the 64u mesh sieve collecting passively at the bottom of the stack....Do you think it's a viable hypothesis? (Claire Buchanan)
- Reply to Claire's question (above): I think your thoughts on the Sieburth paper may certainly be a possibility for the difference and could certainly be mentioned, but I believe there may be a more general principal that applies as well. My gut feel has always been that the more you handle these samples, the greater the loss that will occur, even if the methodology for handling the sample may appear to be more sophisticated than a simpler method. So even if, all things being equal, the statistics on a splitter that has 4 splitting chambers are acceptable, the precision will be sequentially less if a sample is really dense and you need to go to say a 1/64th or 1/128th split, etc. The more sieves, splits, rinses, etc. the greater potential for error. (Fred Jacobs)

Action: points summarized in new Discussion section

# Text about ODU count precision

"The study determined that counts produced with the "new" ODU protocol have variances that are much higher than counts produced with the Versar protocol, hence the ODU counts are less precise." (Draft Executive Summary, page 1, first paragraph.)

"The coefficients of variation in the ODU taxa counts were again larger than those for the Versar counts, indicating that count precision was poorer in the ODU counts (Figure 6)." (Draft Report page 14, third bullet)

# Recommendation:

• Replace text in first sentence with "higher than counts produced with the Versar protocol, although this is expected in the results since Versar is counting more individuals of the smaller taxa." Delete text in second sentence. (Kent Carpenter)

- Replace text in first sentence with "... higher than counts produced with the Versar protocol, hence the ODU estimates of precision are lower." (Elgin Perry)
- Include 1-2 paragraphs in the body of the report summarizing Ray Alden's paper and describing of how the CVS method is intended to change the coefficient of variation for certain kinds of species. (Mary Ellen Ley)
- On the last conference call Kent argued with the statement "ODU counts are less precise". Table 7 indicates that the first part of the sentence is true. If Elgin agrees, I would say something like: "Based on 20(or 10?) pairs of ODU/Versar CVs, there is a significant difference in the paired CVs, with higher CVs at ODU." In Conclusion 1, (p.14), last bullet, keep statement that says that ODU's coefficients of variance are higher than Versar's. (Mary Ellen Ley)

# Comments:

- The concepts of bias, accuracy and precision, the relationship of precision to sample variance, and the relationship of sample variance to raw count numbers were discussed during the April conference call. (Claire Buchanan)
- Kent states in comment 2.B. that the CVS method is an accurate method. This has not been demonstrated. (Mary Ellen Ley)

<u>Action</u>: Sentence changed to "The study determined that counts produced with the "new" ODU protocol have variances that are higher than counts produced with the Versar protocol, hence the ODU estimates of precision are lower." Paragraphs added to the new Discussion section of the report.

# Text about species richness

"Furthermore, the number of taxa identified per sample was on average lower in the ODU counts." (Draft Executive Summary, page 1, first paragraph)

On average, ODU identified fewer unique taxa per sample than Versar (Table 9). This observation suggests that the CVS method as it is currently implemented does not produce more accurate estimates of species richness." (Draft Report, page 9, second paragraph, last bullet)

# Recommendation:

- Replace phrase in Executive Summary with "Furthermore, the diversity measures between the two modified methods are not significantly different although the modified Versar method identified on average more taxa than the ODU counts. However, these additional taxa are mostly the smallest taxa that cannot be reliably counted as mesozooplankton." (Kent Carpenter)
- As already stated above, the additional species appear to be smaller taxa that may be expected to be undercounted in the ODU method. Therefore this statement should not be one of accuracy but simply of consistency between the different counts. I believe the methods as proposed for the round 3 splits will test this more closely. (Kent Carpenter)

# Comments:

• Two particular observations of the split sample results bring into question the usefulness of Margalef's Diversity Index as a bay-wide indicator of community health at this time. First, fewer numbers of mesozooplankton species per sample (species richness) were observed in splits processed with the "new" CVS method (ODU) than in splits processed with the modified pipette method (Versar). Second, estimates of total mesozooplankton abundance obtained with the "new" CVS method are still lower than those obtained with

the pipette method. Species richness is a variable in the numerator of the index's equation and total abundance (number of organisms per sample) is a variable in the denominator. When richness is divided by abundance, as in Margalef's Diversity Index, the resulting proportion does not reflect the lower species richness and lower total abundance of the CVS counts, and the Virginia and Maryland diversity indexes are approximately the same. The Shannon-Wiener, Pielou, and Simpson indices of diversity would be similarly affected because they also rely on measures of species proportional abundance. If the two laboratories had comparable methods and similar raw counts, then the diversity indices could be reliably used (Claire Buchanan)

• Reply to Kent's second bullet: this statement needs to be supported by evidence from the data before it can be incorporated. (Claire Buchanan)

<u>Action</u>: No change made to text of Executive Summary or report text. Paragraph added regarding species richness vs diversity measures in new Discussion section of report.

# Text about discontinuing CVS method in Virginia

"The "old" and "new" ODU counting protocols should be discontinued and a counting protocol patterned after the ICES recommended protocol (Harris et al. 2000) should be instated. Backward comparability with the pre-1998 Chesapeake Bay Program mesozooplankton data will unfortunately be lost in Virginia for most mesozooplankton taxa, but Maryland and Virginia results will become comparable and the CBP monitoring programs should be able to calculate and use multiple, Bay-wide mesozooplankton indicators." (Draft Executive Summary, page 1, first paragraph.)

3. The "old" and "new" ODU counting protocols which are based on the Controlled Variability Sampling method (Alden et al. 1982) should be discontinued and a counting protocol patterned after the ICES recommended protocols (Harris et al. 2000) should be instated. Maintaining the "new" Versar method and "new" ODU method will not yield results that are directly comparable and useful for Bay-wide mesozooplankton indicators. The "new" Versar counting method (Maryland program) has improved Versar's ability to measure species richness, an important Bay-wide indicator, and the "new" ODU counting method (Virginia program) has increased ODU's taxa counts per sample. However, the "new" ODU method still produces split sample results that are significantly different from Versar's results (see above). The Versar method is already very close to the ICES recommended protocols and should be maintained. (Draft Report, page 17)

# Recommendations:

- One last test of the reliability and precision of the ODU CVS method and the Versar stempel pipette method should be undertaken before a final decision is made....
   Recommend the CVS method is eventually adopted by both the Maryland and Virginia programs. (Kent Carpenter)
- Recommend making the following changes to #3 in Conclusions (Mary Ellen Ley):
   3. Differences between laboratories may be due to method bias or technician bias, or both. Further work is needed to determine bias. If bias is shown to be method dependent, one method will be selected for both laboratories. The method that yields comparable results, and the best precision & bias will be selected. Recommendations:

Determine which method is truly biased, i.e., is the CVS method underestimating counts or is the Stempel pipette method overestimating?

Check if CVS method is biased low due to sieving loss. Reanalyze one sample multiple times. Diminishing recoveries of species abundances will indicate loss. Check if CVS method bias due to Folsom splitter. Follow Standard Methods 19<sup>th</sup> edition procedure to verify that the splitter is unbiased and to determine the sampling error introduced by using it.

Analyze a sample of known species identities and abundances with the CVS and Stempel pipettes each method. This comparison should be done within ODU and between ODU and Versar. (Custom made sample)

Check the Stemple pipette method subsampling and sorting bias using procedure in section 2.1.8 of the IPB Handbook Both Versar and ODU need to do this. Determine technician bias by comparing results from the Stempel pipette method performed by both ODU and Versar.

- Remove #3 conclusion and possibly # 4 and #5 (Rick Hoffman)
- I feel it is extremely important that you leave in your "recommendations" as stated in the original report. (Bruce Michael)

- I come to a different conclusion than what is stated in this report, based on the available data and discussions with participants in the review. I believe it is most logical that Versar adopt the CVS method rather than ODU begin using the stempel pipette method employed by Versar. The main reasons for this are twofold. I will summarize these here and explain further below:
  - 1) The split sample tests so far have only established that the Versar method counts more of the smaller zooplankton and not whether ODU undercounts or Versar overcounts. These smaller zooplankton are not reliably counted because of the methods employed in any case and therefore should not be counted on as being important for our purposes.
  - 2) It was pointed out by Fred Jacobs during the 4/11/00 conference call and agreed by everyone (or at least not objected) that Versar should begin using the more common UNESCO recommended field sampling net with a diameter of around .5 m, similar to the one currently used by ODU. Once this new net is employed, Maryland will lose back-compatibility with its data set. If ODU switches to the stempel pipette method, it will also loose back-compatibility with its data set. It makes more sense to loose backward compatibility in only one State. And, since the CVS method is not that much more difficult than the stempel pipette method used by Versar it would not be over-burdensome for Versar to adopt the CVS method. However, I do agree that one last test of the reliability and precision of the ODU CVS method and the Versar stempel pipette method should be undertaken before a final decision is made. I agree that ODU should switch to the Versar stempel pipette method if the new round of split sample tests indicates that the CVS method is substantially less precise than the Versar stempel pipette/folsom splitter method. (Kent Carpenter)
- The CVS method has the advantage of being able to examine fine structure of zooplankton community structure. If the CVS method is eventually adopted by both the Maryland and Virginia programs, as I recommend, many more possibilities exist to identify Bay wide indicators. The stasis or change of composition of the different sieve size classes and their taxonomic components offers many possibilities to examine

abundances and diversity at different trophic levels. This may more clearly identify components of the zooplankton that are important to other trophic levels such as juvenile fishes. With both Maryland and Virginia monitoring these components and both programs examining results, we have greater possibilities for making linkages to both upper trophic levels and water quality in general. I believe this advantage of the CVS should be considered in the report and that consideration be made that all sieve size fractions be reported to the Bay Program as part of normal data submittal. (Kent Carpenter)

- I agree with the report's conclusion (on Page 2-3) that begins with "The "old" and "new" ODU counting protocols should be discontinued." (Fred Jacobs)
- Kent assumes that Maryland would lose backward compatibility if a gear modification to a 0.5 m net were implemented by the Maryland program. When I brought this up on our 4/11/00 call, I meant to imply that we should *consider* making this modification. We would not make such a change until side by side field comparisons between the 0.5 and 0.2 m nets were conducted. If for some reason a systematic bias were to occur (e.g. 0.5 m net consistently gets higher counts than 0.2 m net), we would adjust our historical density estimates accordingly. We would need to ensure that any proposed change will allow for backwards compatibility. (Fred Jacobs)
- Include a section about why bay-wide indicators are important and what we need in the monitoring data in order to ensure useful indicators. (Mary Ellen Ley)
- Reply to Kent's comment # 1) above: see comment by Claire Buchanan under <u>Text about taxa lost by CVS sieving protocol and resulting undercount</u> (above)
- I think the #3 conclusion (i.e. DOU CVS method discontinued) and possible even 4 + 5 (though I think nobody discagrees with these) should be removed from the report for the following reason (Rich Hoffman):

The purpose of the report I thought was to report on the split sample study which developed and tested the success/failure of a "patch". I know you've done a lot of work and the report does a good job of achieving this objective as stated in conclusions 1+2. These final 3 (esp #3) conclusions are actually recomendations based upon your, and others, opinion but not necessarily a direct result of the split study data.

I guess maybe it depends on who is the "Author" of the report. If you alone and it is to represent your analysis alone, then maybe it's ok as is (esp if you move these "conclusions" to a "recommendations" section). If it is a collaborative report (with you as primary leader) then I think it should reflect the other collaborators analysis and agreement. As we know from Kent's submissions, the report does not currently reflect all collaborator opinions, and I don't think I agree with #3 as a "conclusion" supported by the data (as I say above, I think it is a recommendation).

Action: Paragraph on data needs of bay-wide indicators inserted in new Discussion section. Last three "conclusions" changed to "recommendations" (page 15) Changes recommended for #3 by MEL were made (page 15). #4 and #5 left in because there seems to be a consensus on the general ideas. Original text in #3 included in a paragraph in the discussion.

# Possible useful four taxa for long-term comparisons

<u>Recommendation</u>: *Bosmina* is listed as one of the four taxa which may agree between the states. On Table 11, *Bosmina* have a 39.9 percent difference, and Chydorus/chydorids have - 11.42 percent difference. Should chydorus/chydorids be listed as one of the four taxa with less than 20% difference instead of *Bosmina*? (Mary Ellen Ley)

<u>Comment</u>: There were identification problems with the chydorids and barnacle cypris in Maryland prior to 1999, making this species unsuitable for long-term trends. Further exploration of the actual monitoring data (new paragraph in Discussion) is making me rethink the choice of some of the four taxa listed on page 1. (Claire Buchanan)

<u>Action</u>: paragraph in new Discussion section further discussing the usefulness of the four taxa for long-term trends.

# Section entitled "Split Sample Project - Round 1" (Draft Report, page 3)

# Recommendation:

- I believe the general points from Round 1 should be mentioned but that discussion of data that is proven irrelevant should not be included in the main body of the report. Perhaps as an appendix? (Kent Carpenter)
- Since Round 1 data was invalidated, I would downplay quantitative data analysis and interpretation from ODU's Round 1 samples. Qualitative statements are OK, i.e., related to the presence or absence of a species. (Mary Ellen Ley)

Comment: The motorized siever malfunctioned during the first round because of the 'fix' modification, invalidating the round 1 results. The motorized siever that has been used by ODU for the CVS method previously had sieve sizes as follows: 2000, 850, 600, 300, and 200 microns. In order to sample the smaller zooplankton that the stempel pipette samples, and additional sieve chamber with a size of around 75 microns was added to the bottom of the sieve array. This appeared to function normally and the first round of splits was carried out. After the plankton summit, it was noticed that a lot of pressure was building up in the sieve array because of the additional small mesh size that was added. Upon close examination, it was noticed that a small number of the smaller zooplankton were being forced out of the sides of the smallest, added, sieve chamber. This was not readily visible and could easily have gone undetected since the operation looked normal to all who normally operate the motorized siever. When it was detected, we ran a test of the discharge water and determined that a variable number of organisms were being forced out in between the 200 and 75 micron sieve chamber seals. The normal tolerances that worked for the other sieve chambers was not working for the 200 - 75 micron chamber because of the low sieve size and increased water pressure built up in the 75 micron sieve chamber. This problem was fixed when the 75 micron chamber was detached and a 63 micron passive sieve placed underneath as a catch basin for discharge water. However, because of this unexpected malfunction, the results of the first round are invalid and any comparisons between abundances, diversity, and taxonomic make up should be discounted. This is not to say that the first round and the discussions at the plankton summit were fruitless and should be discounted, because many issues were addressed that went beyond the results of the first round splits. (Kent Carpenter) Action: Condense Round 1 section of report.

# Text about Versar counting method

"Versar follows a counting technique patterned after the UNESCO approved method which has been recently affirmed by the International Council for the Exploration of the Sea, ICES (Harris et al. 2000)." (Draft report, page 4, second paragraph, second bullet)

#### Recommendations:

- List the appropriate references. (Kent Carpenter)
- Text should be modified to state something like, "Versar follows a variation of a commonly used counting technique of subsampling using the Stempel pipette method." (Fred Jacobs)

#### Comments:

• There apparently is no such thing as a "UNESCO approved method." Only two UNESCO publications deal with zooplankton methodology (as far as I can tell through several bibliographic searches): UNESCO, 1968 and UNESCO, 1976. Neither of these publications mention subsampling of zooplankton samples using the stempel pipette or the folsom splitter. Therefore, the UNESCO publications do not deal with or approve of a particular subsampling technique.....

The ICES Zooplankton Methodology Manual (2000) is a 684 page book in which one paragraph deals specifically with subsampling methodology. It superficially covers the stempel pipette, folsom splitter, and Kott splitter techniques but does not specifically recommend any one of these methods. It states the coefficient of variation for the stempel pipette and folsom splitter methods and since this coefficient appears to be wider for the Folsom splitter, it could be interpreted as one justification for choosing the stempel pipette method. And, if one examines the studies that are cited in the ICES manual you see conclusion statements such as "For fish eggs the Stempel pipette was most precise and very fast, though it is often impractical for nomral samples because of clogging" and, "For the wild sample, again the Folsom splitter was the most accurate and precise" (Guelpen et al. 1982). A recommendation is not specifically stated in the ICES manual and to state that any method mentioned in this paragraph is somehow ICES approved is making an interpretation that probably extends beyond what the authors intended. Regardless of author intent, since the Versar method employs both a stempel pipette and a folsom splitter, the coefficients of variation cannot be construed to refer to the Versar method. The Versar innovative subsampling combination is not considered in the ICES paragraph dealing with subsampling techniques.

The Versar stempel pipette/folsom splitter combination emphasizes the use of the stempel pipette and therefore can be considered a variation on a stempel pipette method. The CVS method can also be considered a variation on the folsom splitter method since the folsom splitter technique is closely followed and the main difference is that different sieve size fractions within the split are counted. However, both the Versar and ODU methods establish and count dominants and subdominants in different subsamples of the same sample. Since both the stempel pipette and folsom splitter basic methods are mentioned in the ICES manual, both the Versar and ODU methods are more-or-less equally treated in the ICES manual. Although neither are specifically approved or recommended.

The IBP Handbook 17 "A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters" (1984) does specifically recommend the stempel pipette

method. However, Versar does not use the recommended method since they do not follow the minimum prescribed pipette volume of 2.5 to 5.0 ml (Versar uses a 1.0 ml pipette to establish dominants) and they use a combination of stempel pipette and folsom splitter, which is not part of the recommended methodology. And, although methods used in fresh water may be useful for estuarine waters, oceanographic methods are more commonly employed. (Kent Carpenter)

• Reply to Kent's comment: Kent is correct in that UNESCO does not have an approved method for splitting and the text should be modified. We do know that the Stempel pipette has been used in a number of zooplankton programs. In addition to the IBP manual, there are also a number of other citations that can be used to support the use of the Stempel pipette. Weber (1973) describes the use of this method in a published USEPA manual for sampling in surface waters. Frolander (1968) evaluates the method and offers recommendations for improving its reliability. The ICES (2000) document that Kent mentions also discusses the Stempel pipette and indicates a relatively low coefficient of variation of 7-9% when compared to other methods.

I also don't believe it is fair to describe the Versar method as a "hybrid stempel pipette/folsom splitter method". What happens is this - in about 99% of all cases the Stempel pipette is used exclusively. About 1% of the time, the sample is so dense that it cannot be diluted to a workable sample without splitting. In these rare cases, the sample is split with the Folsom splitter, and then the Stempel method is employed. I suggest the text be modified here to state something like, "Versar follows a variation of a commonly used counting technique of subsampling using the Stempel pipette method." (Fred Jacobs)

• Reply to Kent's comment that "Versar does not use the recommended method since they do not follow the minimum prescribed pipette volume of 2.5 to 5.0 ml." Versar seems to have *enhanced* the Stempel pipette method recommended in the 1971 and 1984 IBP Handbooks, i.e. they count 1-2 ml, 5 ml and 10 ml subsamples (see Appendix A in Report).

The precision values given in Table 4.11 of the ICES Manual (pg 151) can be directly applied to the results of the Versar method when the Folsom splitter was not used because organisms were randomly distributed at the time of subsampling. (Claire Buchanan)

<u>Action</u>: Text modified to read "Versar follows a variation of a commonly used counting technique of subsampling using the Stempel pipette method." Relevant references for the laboratory method currently used by Versar were requested from Fred Jacobs and William Burton.

#### Text regarding which ODU staff counted splits in Round 1

"George Mateja, the senior ODU counter of the ODU staff, counted the 24 Virginia split samples." (Draft Report, page 3)

Recommendation: change incorrect statements regarding which ODU staff counted the Round 1split samples.

- This is not true. It was well known at the time, and discussed during the plankton summit that the original Virginia split samples were read by the two senior counters (Miebert and Crock) and the lab supervisor (Mateja). (Kent Carpenter)
- Table 6 contains many poor assumptions, inaccuracies, and conclusions and should be removed altogether. First, it was well know at the time that Round 1 was counted by the three senior ODU counters. Some of these counts actually compare Crock versus Crock. (Kent Carpenter)
- The author of this report was under the impression that the ODU laboratory supervisor, George Mateja, was the sole counter of the Virginia split samples in Round 1. This misunderstanding was not corrected in the minutes of the "Plankton Summit" circulated in September 1998. It was not corrected in discussions of the Round 1 First Five split sample results. It was not corrected in the draft findings of "Round 2 First Ten" emailed to the zooplankton principal investigators and staff on February 1, 1999 and discussed in a subsequent conference call. This delay in correcting an important misunderstanding led directly to the author making erroneous statements and incorrect conclusions in the report (Draft Report pages 3, 8, 9). (Claire Buchanan)

Action: Text and conclusions modified.

Explanation of taxonomic differences in Round 1 and Round 2 repeat counts done by ODU "Differences in the copepod and cladoceran species listed by the ODU Round 1 counter, the ODU Round 2 counter, and the Versar counter suggests there may be species identification discrepancies that should be investigated as well in the se taxonomic groups." (Draft Report, page 8, last bullet in first paragraph).

"Visual comparison of individual taxa counts in the Round 2 first ten split samples suggest that differences may also be occurring between the senior and junior ODU counters (Table 6)." (Draft Report, page 9, fourth bullet in second paragraph)

Headers in Table 5. (Draft Report, page Table-6)

# Recommendation:

• Remove erroneous conclusion and associated text (Kent Carpenter)

# Comments:

• The most logical explanation for most taxonomic differences stems from the malfunction of the motorized siever in the first Round. Contamination is an extremely remote possibility, but this possibility also could have occurred during Versar counts with equal probability. The comparisons clearly show that most problems with the taxonomic differences are most likely due to small taxa being lost from the seal between the 200 micron and 72 micron chambers. (Kent Carpenter)

<u>Action</u>: Text in paragraphs relating to this item were changed, and conclusions revised. Footnote inserted and reiterated later.

# Taxa misidentifications

- Versar technical staff previously misidentified barnacle cypris (eggs) as ostracods at high salinity stations.
- The junior ODU staff had been misidentifying *Eurytemora* as *Temora* at some freshwater stations. This error was most likely due to inexperience, and the taxonomist presently can identify the difference between the genera. (Draft Report, page 10, first paragraph)

#### Recommendation:

- Please add after the sentence about the Versar technical staff (first highlighted point above): "This error was most likely due to inexperience, and the taxonomist presently can identify the difference between these two major zooplankton components." (Kent Carpenter)
- Change "junior" to "senior" staff member. (Kent Carpenter)
- I don't think we need to speculate about inexperience of ODU or Versar personnel. We can just state the taxonomic groups that Versar and ODU staff misidentified, and indicate that measures were taken to correct the problems. (Fred Jacobs)

#### Comments:

- First, the ODU staff that misidentified this was a senior staff member (not a junior member as interpreted here) that was not used to counting freshwater stations (we specialize counting in the lab according to salinity zones). True, it was probably due to inexperience with freshwater taxa. However, this same ODU senior staff member also was the one that pointed out that the Versar technical staff member was misidentifying barnacle cypris eggs as ostracods. If you are going to assert that the ODU staff mistake was due to inexperience, it would be unbiased to also assert the same for the Versar technical staff. (Kent Carpenter)
- The bullets were taken almost verbatim from "Appendix C: Letter from Versar to Maryland Department of Natural Resources Following March 10-12, 1999 meeting at Old Dominion University." (Claire Buchanan)

Action: Text changed according to Jacobs recommendation.

#### Specimen Archive

"Specimen Archive. Each laboratory would begin to assemble a reference collection of all the species encountered during regular sample analyses. Specifically, 2 or more individuals of each species (and sex if possible) would be picked and placed in a sample vial for that species. This could eventually become a long-term reference collection to be compared and shared by both laboratories." (Draft Report, page 13)

"5. A record of the mesozooplankton taxa identified in the CBP zooplankton monitoring program should be maintained in both laboratories (e.g. a type specimen collection, a photographic record). Laboratory differences in taxonomic identifications can be reconciled during side-by-side comparisons and through the assembly of a photographic or type specimen collection for Chesapeake Bay mesozooplankton." (Draft Report, page 15)

<u>Recommendation</u>: Allocate resources to create a taxonomic guide to zooplankton of the Chesapeake Bay, to ensure that monitoring programs identify taxa the same. (Kent Carpenter) <u>Comment</u>:

• I think this is inadequate. It is important to standardize taxonomy between the ODU and Versar programs. Standardization of taxonomy should clearly be one of the most important goals of any future mesozooplankton common method between Maryland and Virginia. I disagree that a reference collection of zooplankton species should be the main component to help with this standardization. A reference collection should be made as a component of this coordination. However, the best way to ensure that this taxonomy is standardized is for both programs to use the same taxonomic guide to zooplankton of the Chesapeake Bay. I believe that one priority should be that resources be allocated to

achieve this. I have offered to help and welcome any combination of ODU, Versar, or ODU and Versar collaborating to complete this guide. (Kent Carpenter)

Action: Wording was changed to better reflect these comments.

### Correction factors

"Correction factors. Claire Buchanan will review all the split sample results and construct a table of conversion factors for common mesozooplankton species. These conversion factors will be used on the older, "pre-patch" ODU and Versar data for the purpose of calculating Bay-wide indicators." (Draft Report, page 13)

# Comment:

- Don't like correction factors. (Mary Ellen Ley)
- This approach is looking very weak at this point. (Claire Buchanan)

<u>Action</u>: include some discussion of the doubtfulness of using this approach in the new Discussion section.

# Quality assurance counts

"4. Quality assurance counts within each laboratory and between laboratories should be rigorously maintained, documented, and periodically reviewed to ensure comparable, high quality mesozooplankton counts. Quality assurance procedures should be maintained in each laboratory to ensure adequate taxonomic training of new technical staff. Quality assurance (repeated) counts for each laboratory should be regularly submitted to the states, the Chesapeake Bay Program or their designees for independent analysis. Regular site visits between the two states' technical staffs should be carried out to ensure comparable interstate taxonomy. A split sample study should be done annually for at least the next few years to ensure interstate count comparability." (Draft Report page 15)

<u>Recommendation</u>: Institute a common QA/QC plan. (Kent Carpenter) Comment:

• I strongly agree... I would recommend that whatever method is commonly adopted by ODU and Versar, that common QA/QC plans be followed. This should follow a thorough QA/QC review and a plan adopted that is meaningful and practical given budgetary constraints. (Kent Carpenter)

Action: Recommendation altered to reflect comment.

#### General comments on Conclusions

- I agree with the conclusions, assuming the relevant wording changes I suggested earlier in this review are implemented. (Fred Jacobs)
- How about: 1. Inter-laboratory split sample comparisons between ODU and Versar indicate that the laboratories do not produce comparable abundance data for most species. (Keep bullets the same.) (Mary Ellen Ley)

Action: Changed text of #1 to MEL's recommendation.

<u>Field sampling method</u> (Although not directly a part of the split sample study, this issue was discussed several times during the course of the study.)

- Versar should begin using a standard field sampling net which will make its future data incompatible with past data. This point is not really a disagreement with the current report but should be included in the report as discussed in the 4/11/00 conference call. UNESCO (1968) clearly recommends a plankton net opening of around 50 cm for the size of mesozooplankton that we are intending to sample. And, as far as I can tell from the literature, and as Fred Jacobs asserted in the 4/11/00 conference call, a 50 cm diameter net opening is the most commonly used method and the Maryland program should begin using this sampling method. This change in sampling net will make their future data incompatible with past data.. (Kent Carpenter)
- Kent is correct in that a 50 cm mouth opening net is the most common net used but there is certainly precedent for using 10 cm, 20 cm, 60 cm, 1m and 2m nets. The BLM zooplankton offshore programs of the 1970s and 1980s used 20 cm Bongos, 60 cm opening/closing Bongos, and 1 m nets, all for specific sampling objectives. In most cases, the larger the mouth opening, a higher, more accurate estimate of density will occur. This is because of reduced avoidance with larger mouth openings and presumably greater volumes of water sampled. Why don't we then just sample with 1 or 2 m nets? The answer is the: difficulty of handling such gear (especially from small boats), amount clogging in estuarine waters, and the excessive amount of laboratory time it would require to process, split and enumerate samples. It just would not be a prudent way of spending our limited resources.

Furthermore, many of the gear studies have been done with oceanic plankton such as euphausiids and large copepods, which have greater avoidance capability than estuarine zooplankton, largely dominated by copepods in the 1mm size range, and even smaller cladocerans. Other factors such as tow speed and tow length are generally considered to be more important than size of mouth opening. For example, Wiebe (1970, 1971, 1972) conducted a series of gear studies in the 1970s. He (Wiebe 1972) concluded that increasing mouth opening from 25 cm to 1 m improved the precision of his density estimates by 15 to 19% (averaged across three tow lengths), but increasing the tow length from 500 m to 2000 m improved precision by 45% (averaged across four mouth opening sizes). There was much less of a difference in precision for nets of any size mouth opening in longer tows. Both the 0.25 and 1 m nets that were towed 2,000 m had greater average precision than either net towed at 500 m. His conclusion was that increasing tow length improves precision of replicates and provides better estimates of the relative proportions of species than does enlarging net diameter. The point is also made that it is not necessarily the volume filtered that is important but the ability to integrate across patches that can be achieved by longer tow lengths. Versar does extend their tow times in an attempt to integrate across patches.

For these reasons, I am not convinced that Maryland would achieve much improvement in precision by switching to the larger mouth opening in the estuarine environment, although it is certainly possible. If Maryland does ultimately change to a 50 cm net, we should make sure that tow distances are relatively constant between the Versar and ODU programs.

When we started the Plankton Monitoring Program in 1985 there was no Virginia Zooplankton program. The other large scale zooplankton monitoring program that was conducted for Chesapeake Bay (from 1971-1974) used a 20 cm Bongo and, thus,

- provided a good basis for comparison. Other factors we were concerned about included the high degree of turbidity in certain Maryland tributaries, and types of vessels that would be available for tributary sampling. For these reasons, the 20 cm net was selected. (Fred Jacobs)
- If Versar uses a smaller diameter net, results *could* be affected significantly. Kent assumes that they will be affected, but to really know, a side by side comparison would have to be done. (Mary Ellen Ley)

Action: None

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